# The Effect of Soil Properties on Metal Bioavailability: Field Scale Validation to Support Regulatory Acceptance

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| REPORT DOCUMENTATION PAGE | *FORM APPROVED*<br>*OMB NO. 0704-0188* |
|---|---|

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

| 1. REPORT DATE *(DD-MM-YYYY)*<br>23-04-2013 | 2. REPORT TYPE<br>Technical Report | 3. DATES COVERED *(From – To)*<br>September 2005 – April 2013 |
|---|---|---|

| 4. TITLE AND SUBTITLE<br>The Effect of Soil Properties on Metal Bioavailability: Field Scale Validation to Support Regulatory Acceptance<br>ESTCP ER-0517 | 5a. CONTRACT NUMBER |
|---|---|
| | 5b. GRANT NUMBER |
| | 5c. PROGRAM ELEMENT NUMBER |
| 6. AUTHOR(S)<br>Amy Hawkins, NAVFAC EXWC; Mark Barnett, Auburn University; Nick Basta, Elizabeth Dayton, Roman Lanno, Ohio State University; Stan Casteel, University of Missouri, Phil Jardine, University of Tennessee; Kaye Savage, Wofford College | 5d. PROJECT NUMBER |
| | 5e. TASK NUMBER |
| | 5f. WORK UNIT NUMBER |

| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)<br>Click here to enter text | 8. PERFORMING ORGANIZATION REPORT NUMBER |
|---|---|

| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)<br>Environmental Security Technology Certification Program | 10. SPONSOR / MONITOR'S ACRONYM(S)<br>ESTCP |
|---|---|
| | 11. SPONSOR / MONITOR'S REPORT NUMBER(S) |

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Distribution statement A: Approved for public release; distribution is unlimited.

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

The technical objectives of the investigation were: (1) To provide validation that the relationships between soil properties and *in vitro* bioaccessibility methods can serve as a screening tool for estimating *in vivo* toxic metal bioavailability in DoD soils; (2) To provide DoD with a scientifically and technically sound method for estimating human and ecological risk associated with metal contaminated soils in place of or as justification for more-detailed, site-specific bioavailability (e.g., animal dosing), and (3) to promote the use of *in vitro* methods in human health and ecological risk assessments through the upfront involvement of end-users and regulators and the subsequent dissemination of the results of the study in peer-reviewed journals.

**15. SUBJECT TERMS**
Bioavailability, metals, soil, bioaccessibility, ecological risk, arsenic, cadmium, chromium, lead

| 16. SECURITY CLASSIFICATION OF:U | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON<br>Amy Hawkins |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | 19b. TELEPHONE NUMBER *(include area code)* |
| U | U | U | SAR | 275 | 805-982-4890 |

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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# Preface

Most of the technical objectives, methods, results, discussion, conclusions, and recommendations of this study are detailed in Appendices A-F, which were written as stand-alone manuscripts for submission as peer-reviewed publications. Publication in peer-reviewed journals is needed to disseminate and ultimately facilitate the results of this study by site managers. In addition, publication in peer-reviewed literature is crucial to ensuring regulatory and community understanding and acceptance of the scientific results. Appendix A describes the speciation of metals and metalloids in soils that we sampled from U.S. military facilities as measured using synchrotron x-ray absorption techniques. Appendix B describes models for predicting metal toxicity and bioaccumulation in soil invertebrates, with a focus on the validation of laboratory models using metal-contaminated field soils. Appendix C describes a phytoaccumulation study, where comparison of the actual contaminant phytoaccumulation from bioassays with predicted toxicity from *in vitro* models were used to quantify the ability of *in vitro* models to predict actual phytoaccumulation in field DoD soils. Appendix D describes an *in vivo* study of As, Pb, and Cd using the juvenile swine model. Appendix E describes the ability of soil properties and *in vitro* extraction methods to predict bioavailability and bioaccessibility of As, Pb, and Cd in soil. Finally, Appendix F describes the soil properties and metal(loid) concentrations from the soils that we utilized. Liberal citations to these Appendices are made in the report itself, and an effort was made to minimize unnecessary duplication of material between the body of the report and the Appendices themselves. However, some duplication of material was used as needed to allow the report and each of the Appendices to be read as independent documents.

# Executive Summary

The Department of Defense (DoD) faces a potentially daunting task of remediating thousands of metal-contaminated sites within the U.S. and its territories that contain unacceptable levels of the toxic metal(loid)s As, Cd, Cr, and Pb.  With the exception of Pb contaminated soils, human health and ecological risk drivers have prompted EPA to assume that the total soil metal concentration is 100% bioavailable. Previous SERDP funded research (CU-1166 and CU-1210), however, has shown that the ubiquitous metal-sequestering properties of soil can significantly lower the bioavailability and risk of heavy metals to human and ecological receptors.  This investigation brought together regulators, EPA, end-users, and scientists to demonstrate the applicability of these concepts by showing that simple, readily available soil properties can often be used to predict the bioavailability of As, Cd, Cr, and Pb with a reasonable level of confidence. We have shown that *in vitro* methods can often be used for risk assessment of toxic metals in soil by comparing *in vitro* and *in vivo* metal bioavailability studies.

The technical objectives of the investigation were: (1) To provide validation that the relationships between soil properties and *in vitro* bioaccessibility methods can serve as a screening tool for estimating *in vivo* toxic metal bioavailability in DoD soils; (2) To provide DoD with a scientifically and technically sound method for estimating human and ecological risk associated with metal contaminated soils in place of or as justification for more-detailed, site-specific bioavailability (e.g., animal dosing), and (3) to promote the use of *in vitro* methods in human health and ecological risk assessments through the upfront involvement of end-users and regulators and the subsequent dissemination of the results of the study in peer-reviewed journals.

Performance Objectives 1 and 2 involved testing the bioavailability screening tools developed in our earlier SERDP studies, which correlate chemical speciation, bioaccessibility, bioavailability, and toxicity of metals (As, Cd, Cr, Pb) in DoD soils as measured by biological models used to evaluate ecological risk (e.g., plants, earthworms) and human risk (e.g., immature swine model) Only three sites were considered for the *in vivo* swine dosing studies due to the experimental cost.  The use of *in vitro* ecological models were further verified by comparison with *in vivo* ecological bioassay studies of eleven DoD soils (eleven contaminated, eleven control).

Metal Speciation An important first step was characterizing the molecular-level speciation of the metals in the soil with the use of X-ray absorption spectroscopy.  Synchrotron X-ray fluorescence microprobe mapping, microbeam X-ray absorption spectroscopy, and bulk sample X-ray absorption spectroscopy were used to determine the oxidation state and molecular coordination environment of As, Cr, and Pb in eleven study soils with variable soil properties. *In vivo* swine dosing trials to determine metal bioavailability, *in vitro* gastrointestinal studies to determine metal bioaccessibility, soil extraction procedures and soil properties used to predict metal bioavailability to plant and soil invertebrates and ecological bioassay studies were also performed on the same set of soils.  Findings from synchrotron X-ray studies indicated that Pb is adsorbed as divalent ions or present as organic complexes, rather than in crystalline compounds. Chromium and As are present in their more stable and less toxic inorganic forms, Cr(III) and As(V), except in soil from the Naval Complex at Pearl Harbor, where both As(III) and As(V) are present. Arsenic is bound to iron oxides in the Concord and Pearl samples, and to aluminum oxides in the Hilo soil sample. Arsenic-bearing soils may require more site-specific approaches

to remediation. Lead was not bound in sulfide phases that would be considered stable, meaning that most of the Pb-O in the soils may be liberated under acidic conditions (i.e., in the stomach).

<u>Bioaccumulation and Toxicity Models</u> Metal bioaccumulation and toxicity to soil invertebrates (*E. andrei, En. crypticus, F. candida*) were examined in ESTCP metal-contaminated soils (with paired reference site soils) comprising a wide range of physical and chemical characteristics and metal levels. The predictive ability of a number of different models relating soil properties to oligochaete metal bioaccumulation as a screening tool for estimating metal bioavailability in soils was examined with the intent of validating some of these models for predicting metal bioaccumulation in soil-dwelling oligochaetes.

Key elements for predicting bioaccumulation of metals by soil invertebrates include total metal concentration in the soil, soil physicochemical characteristics, and time. In this study, we examined the application of various models, with varying degrees of success, in predicting the bioaccumulation of metals by earthworms from ESTCP soils. The models can be divided into three categories: 1) Metals for which a large number of models exist in the literature (e.g., Pb, Cd); 2) Metals for which few models exist in the literature (e.g., Cr, Ni); and, 3) Essential metals (e.g., Cu, Zn).

When applying literature-based metal bioaccumulation models to assess Cd and Pb bioaccumulation by earthworms in metal-contaminated field soils, 98% of the variability in earthworm Cd concentrations could be predicted by a model comprising total soil Cd, organic matter content, and soil pH, while 95% of the variability in earthworm Pb concentrations could be predicted by a model including total soil Pb and soil pH. However, both these models over-predicted metal bioaccumulation (Cd Root Mean Square Error (RMSE) - 106%; Pb RMSE - 272%) so their use in predicting bioaccumulation may be limited. A large portion of the variability in the tissue concentrations of As ($R^2$ - 90%), Cr ($R^2$ - 77%), and Ni ($R^2$ - 88%) could be estimated by their concentrations in soil. Even though just a few bioaccumulation models exist for these metals, the models for As (RMSE - 24.2%) and Cr (RMSE - 13.6%) provided acceptable predictions of metal uptake, while the Ni model severely over-predicted uptake (RMSE - 689%). However, for the essential metals Cu and Zn, total soil concentrations combined with soil properties provided a reasonable prediction of tissue concentrations for Cu (RMSE - 24.7%) but not for Zn (RMSE - 590%). A model relating bioaccumulation factor (BAF) of Cd to soil properties provided acceptable predictions of Cd BAFs by *En. crypticus* from ESTCP soils (RMSE - 20%) while no relationship was evident between BAFs and observed metal burdens for Pb and Zn.

Models developed relating 0.5 M $Ca(NO_3)_2$-extractable Cd and Pb to earthworm metal residues did not provide a better prediction of Cd and Pb concentrations in earthworms exposed to ESTCP soils than models selected from the literature that predicted earthworm metal concentrations based upon total metal levels and soil physicochemical characteristics. Models incorporating toxicokinetics of metals were only available for Cd and provided reasonable estimates of Cd concentrations in earthworms (RMSE - 19%). These results indicate that there are no models for a specific metal that would provide good predictions of metal bioaccumulation in all soils and situations.

The capability of soil property/soil extraction models to predict soil invertebrate bioaccumulation of metals from contaminated soils is summarized as follows.

**Table ES-1. Summary of the Prediction of Metal Bioaccumulation by Earthworms (*Eisenia fetida*) or Potworm (*Enchytraeus crypticus*) using Soil Property or Soil Extraction Data**

| Approach | Metal | Model | Summary and ability to predict metal body burdens |
|---|---|---|---|
| Soil properties | As | $\ln As_{ew}=0.9884*\ln As_s - 1.747$<br>Sample et al. 1998 | Based on total As levels; $R^2=0.90$; under predicts 0.8-16-fold, most soils 0.8-3.3 fold; RMSE = 24.2% |
| | Cd | $\ln Cd_{ew} = 6.018 + 0.787 * \ln Cd_s - 0.106 *OM - 0.402 * pH$        Ma et al. 1983 | Based on total Cd, organic matter, pH; $R^2=0.98$; over predicts 3.8-11.3-fold; only eight data points above DL; RMSE = 106% |
| | Cr | $\log Cr_{ew}=0.69*\log Cr_s -1.05$<br>Peijnenburg et al. 1999a | Based on total Cr; $R^2=0.73$; under predicts 0.8-7.4-fold; RMSE == 13.6% |
| | Cu | $\log Cu_{ew}=0.435*\log Cu_s +0.39$<br>Morgan and Morgan 1988 | Based on total Cu; $R^2=0.45$; under predicts 1.3-5.2-fold; RMSE = 24.7% |
| | Ni | $\log Ni_{ew}=0.98*\log Ni_s +0.67$<br>Neuhauser et al. 1995 | Based on total Ni; $R^2=0.88$; over predicts 11-95-fold; RMSE = 689% |
| | Pb | $\log Pb_{ew} = 2.65+0.897 *\log Pb_s-3.56*\log pH$<br>Corp and Morgan 1991 | Based on total Pb and pH; $R^2=0.95$; over predicts 0.5-25-fold; RMSE = 272% |
| | Zn | $\log Zn_{ew}=1.45*\log Zn_s +0.42$<br>Peijnenburg et al. 1999a | Based on total Zn; $R^2=0.62$; under predicts 1.3-5.2-fold; RMSE = 590% |
| | Cd | $C_w = 9.32 *e^{-0.008*28} + Cd_s *0.052/0.008*(1-e^{-0.008*28})$<br>Yu and Lanno 2010 | Based on Cherry Point and McLellan soils where total Cd is same as model concentration, one prediction is the same as observed and one is 2-fold higher; with all 8 data points – RMSE = 19% |
| Calcium Nitrate Extraction | Cd | $\log Cd_{ew} = 0.27*\log Cd_{Ca(NO3)2} + 2.1$<br>$R^2 = 0.66,$ | Only two soils – Cherry Point, McLellan – with total extractable Cd levels; over predicted earthworm Cd 3-6.8-fold; RMSE = 111% |
| | Pb | $\log Pb_{ew} = 0.32\, Pb_{Ca(NO3)2} + 97$<br>$R^2 = 0.39, P=0.008$ | Only five soils with extractable Pb; over predicted 1.1-3.6-fold; RMSE = 161% |
| | Zn | $\log Zn_{ew} = 0.02\, Zn_{Ca(NO3)2} + 2.12,$<br>$R^2=0.084, P=0.21$ | Only four soils with extractable Zn; under predicted 1.3-2-fold; RMSE = 101% |

| BAF - Soil properties *En.crypticus* | Cd | log BAF=1.17-0.92*log Clay Peijnenburg et al. 1999b | Only six soils where BAF could be calculated; acceptable under-prediction; RMSE = 21% |
|---|---|---|---|
| | Pb | log BAF=0.35-0.36*pH Peijnenburg et al. 1999b | No relationship |
| | Zn | log BAF =3.47-0.46 *pH-0.67*log $Al_{ox}$ Peijnenburg et al. 1999b | No relationship |

<u>Plant Bioaccumulation</u> Contaminant phytoaccumulation was also determined from plant bioassays for soils from eleven study sites. For ecological risk estimates, metal phytoavailability was estimated from soil-property driven multiple regression models developed using bioaccumulation data from two previous study studies.  A separate approach involved the use of soil extraction methods, used to estimate metal(loid) phytoavailability, to predict contaminant phytoaccumulation. Regression models developed using bioaccumulation data from a previous study sponsored by the National Center for Environmental Assessment and SERDP CU-1210 were used to predict contaminant phytoaccumulation in the study soils. Comparison of the actual contaminant phytoaccumulation from bioassays with predicted toxicity from *in vitro* models were used to quantify the ability of *in vitro* models to predict actual phytoaccumulation in field DoD soils. This was the basis for validation of the soil property or soil extraction methods for field DoD soils.  The predictive capability required by a soil property/soil extraction models depends on the degree of accuracy of contaminant phytoaccumulation determined by the risk assessor.   With some exceptions, both methods were able to predict phytoavailability at RMSE<35% of the measured contaminant tissue value.  In general, soil property models were predictive of tissue As, Cd, and Pb.  Exceptions were Deseret for As (ryegrass), Hill for Cd (lettuce), and Portsmouth for Pb.  In general, the predictive capability of soil extraction methods was adequate to excellent with the exception of Hill for Cd (lettuce) and Portsmouth for Pb.

 The predictive capability of soil property / soil extraction models to predict plant phytoaccumulation is summarized as follows.

**Table ES-2.** Summary of the Prediction of Contaminant Phytoaccumulation using Soil Property or Soil Extraction Soil Data

| Approach | Model or Soil Extraction | Ability to Predict Tissue As | | Ability to Predict Tissue Cd | | Ability to Predict Tissue Pb | |
|---|---|---|---|---|---|---|---|
| | | Lettuce | Ryegrass | Lettuce | Ryegrass | Lettuce | Ryegrass |
| Properties | MLR | 4† Concord Over , 5x‡ | 4 Deseret Over, 80x | 4 Hill Under, 1.7x | 4 | 7 Portsmouth Over, 1.3x ORNL Under, 1.3x | 7 Portsmouth Under, 1.2x |
| | RR | 4 | 4 Deseret Over, 80x | 4 Hill Under, 1.7x | 4 | 7 Portsmouth Over, 2x ORNL Over, 2x | 7 Portsmouth Over, 1.7x |
| Soil Extraction | Pore water | 3 | 3 All sites Over, 2x | 3 | 3 Hill Under, 1.6x | 4 Portsmouth Under, 4x | 4 Portsmouth Under, 3.3x |
| | Mehlich 3 | 4 | 4 all sites Over, 2x to 5x | NA | NA | NA | NA |
| | Calcium Nitrate | NA | NA | 3 Hill Under, 10x | 3 Hill Under, 4x | 4 Portsmouth Under, 2x | 4 Portsmouth Under, 2.5x |

† Number of contaminated soils evaluated.

‡ Over prediction of tissue As concentration by a factor of five

<u>In Vitro Testing</u> One of the main objectives of the project was to determine the ability of *in vitro* gastrointestinal methods (i.e., bioaccessibility methods) to predict measured contaminant bioavailability in contaminated soils from study sites. Equations used to predict bioavailability from bioaccessibility methods are available for Pb and As. Relative bioavailable Pb was determined for the Portsmouth soil in our study. The Physiologically Based Extraction Test (PBET) methods (pH 1.5 and 2.5) were able to accurately predict *in vivo* relative bioavailability (RBA) for the Portsmouth soil. The predicted RBA for the PBET method at pH 2.5 was closer to actual *in vivo* RBA than pH 1.5. However both methods predict RBA Pb within the 90% confidence interval. The Ohio State University *In vitro* Gastrointestinal Method (OSU IVG) method i*n vitro* bioaccessible (IVBA Pb) was very close to the *in vivo* RBA Pb. However, information on the ability of the OSU IVG method to predict RBA Pb is very limited whereas in depth validation studies have been conducted for the relative bioaccessibility leaching procedure (RBALP i.e., PBET) method. These results support the use of the PBET method at pH 1.5 and 2.5 to accurately predict *in vivo* RBA Pb. Future validation studies where this approach is expanded from the Portsmouth soil to other DoD soils will increase the confidence of using *in vitro* methods to predict *in vivo* RBA Pb.

**Table ES-3.** Comparison of Measured and Predicted RBA Pb for the Portsmouth Soil

| | | Predicted Pb RBA | | | | |
|---|---|---|---|---|---|---|
| Measured Pb RBA, % | | PBET pH 1.5 | | PBET pH 2.5 | | OSU IVG pH 1.8 |
| Mean | 90 % CI† | IVBA, % | RBA, % | IVBA, % | RBA, % | IVBA, % |
| 99 | 70 - 127 | 83.3 | 86.9 | 80.4 | 106.2 | 102.5 |

† CI = confidence interval

Results from our study show both the OSU IVG and Solubility Bioavailability Research Consortium (SBRC) method were able to predict RBA As in the Deseret soil. The predicted RBA As by all methods ranged from 12.2 % to 16.2%, which is comparable to the *in vivo* RBA As of 14%. Further validation studies of these methods for other contaminated soils from different DoD contaminant sources are warranted. A study investigating the relationship between *in vitro* IVBA Cr and *in vivo* RBA Cr has not been reported. Thus, it was not possible to evaluate the ability of bioaccessible Cr to predict *in vivo* RBA Cr. In our study, a novel immature swine dosing model was used to determine the *in vivo* RBA Cr for the McClellan soil. RBA Cr was 107% with a 90% confidence interval ranging from 76% to 169%. *In vitro* IVBA Cr PBET method, used to measure bioaccessible Cr at pH 1.5 and at pH 2.5, was 10.1% and 19.0%, respectively. The *in vitro* IVBA values were much lower than the *in vivo* RBA Cr. Further research is needed before IVBA can be used to predict *in vivo* RBA Cr.

**Table ES-4.** Comparison of Measured and Predicted RBA As for the Deseret Soil

| | | Predicted As RBA | | | | | |
|---|---|---|---|---|---|---|---|
| Measured As RBA, % | | OSU IVG gastric | | OSU IVG intestinal | | SBET gastric | |
| Mean | 90 % CI† | IVBA, % | RBA, % | IVBA, | RBA, % | IVBA, % | RBA, % |

| | | | | % | | | |
|---|---|---|---|---|---|---|---|
| 14 | 13-15 | 8.45 | 15.0 | 8.47 | 16.2 | 10.6 | 12.2 |

† CI = confidence interval

In general, all of the in vitro methods predicted in vivo RBA As with 90% confidence.

The ability of soil properties to predict As and Cr bioaccessibility (IVBA) was dependent on the contamination source.  In general, IVBA As measured by PBET and OSU IVG could be predicted from measured soil Fe properties including Feox or CBD Fe for soils where arsenical pesticide was the contaminant source.  However, soil properties of the Deseret soil, where mine tailing was the contaminant source, was not predictive of the measured IVBA As.   This finding suggests arsenic may occur as discrete minerals from the mining operation.  It is likely the insoluble As minerals in the mining waste did not appreciably dissolve and react with soil components.  Therefore, its chemical speciation and IVBA solubility will depend on the mining waste mineral not soil property.

The ability of soil properties (i.e., clay, organic and inorganic carbon) to predict and Cr bioaccessibility (IVBA) was dependent on the contamination source. Good agreement between the measured IVBA Cr and predicted IVBA Cr was found for Hill and McClellan soils.  Poor agreement between the measured IVBA Cr and IVBA Cr predicted by soil properties was found for the Cherry Point soil.  Differences in Cr chemical speciation in soil may offer an explanation. Water or wastewater treatment was the contaminant source for the Hill and McClellan soils. Incinerator ash was the contaminant source for the Cherry Point soil.

Summary of  Soil Properties to Predict Metal Bioavailability
Soil properties, able to predict metal (bio)availability for several contaminated soils in this study, are summarized in the following table.  At a minimum, soil property information needed from a site investigation for all contaminants studied are soil pH, clay content, organic C, inorganic C, reactive Fe and Al  (FEAL, Feox and/or CBD Fe).  Other properties not studied that will affect ecological endpoints include soil salinity and the presence of other toxicants.

**Table ES-5.** Summary of Soil Properties to Predict Metal Bioavailability

| | Contaminant | | | |
|---|---|---|---|---|
| | Pb | As | Cr | Cd |
| Human Soil Ingestion Bioaccessibility | Not evaluated | Feox and FeCBD | Clay content, total organic C, inorganic C | Not evaluated |
| Plant accumulation Lettuce | pH, OC, FEAL | pH, OC, FEAL | Not evaluated | pH, OC, FEAL |
| Plant accumulation Ryegrass | pH, OC, FEAL | pH, OC, FEAL | Not evaluated | pH, OC, FEAL |
| Soil Invertebrates | pH | Total metal | Total metal | pH, OM |

These properties will **not** predict metal bioavailability for all soils. A major finding of this study is the contaminant source and likely speciation greatly affects the ability of soil property to predict metal bioavailability. Metal bioavailability was not able to be predicted for several soils where the contaminant source was unweathered mining waste or discrete inorganic mineral forms such as coal ash. Soil properties should NOT be used to predict contaminant bioavailability in these soils. More research on contaminant source and speciation is needed to determine when soil properties can provide an accurate assessment of metal bioavailability. Currently research is in progress, including research funded by SERDP (i.e., ER-1742) to determine the relationship between As speciation and ability to predict As bioavailability to humans.

Summary of Soil Extraction Methods to Predict Metal Bioavailability
Soil exaction methods, able to predict metal (bio)availability for several contaminated soils in this study, are summarized in the following table. Both PBET and OSU IVG were able to very accurately predict RBA As and Pb but for only for 1 soil each. The number of soils evaluated were very limited because of cost constraints associated with in vivo dosing trails required to measure contaminant RBA. More research is needed to evaluate the ability of these methods to predict RBA Pb and RBA As on other contaminated soils.

Soil pore water was able to predict plant tissue concentration of Pb, As, and Cd. Soil extraction with 0.1 M $Ca(NO_3)_2$ was able to predict cationic metal contaminants(i.e. Pb, Cd) but was not evaluated for anionic As contamination. The ability of simply water or dilute calcium nitrate to predict phytoavailable contaminant suggests high solubility of these contaminants in soils. Thus, it is likely that with 0.1 M $Ca(NO_3)_2$ would have also been a good predictor of plant As. However, two cautions should be heeded. The accuracy of these extraction methods to predict plant tissue contamination was limited to ± 35%. Similarly to metal bioaccessibility results, metal bioavailability was not able to be predicted for several soils where the contaminant source was unweathered mining waste (i.e. Deseret) or discrete inorganic mineral forms such as coal ash (i.e. Cherry Point). Soil extraction methods listed in the summary table should NOT be used to predict contaminant bioavailability in these soils. More research on contaminant source and speciation is needed to determine which soil extraction methods can provide an accurate assessment of metal bioavailability.

**Table ES-6.** Summary of Soil Extraction Methods to Predict Metal Bioavailability

|  | Contaminant |  |  |  |
|---|---|---|---|---|
|  | Pb | As | Cr | Cd |
| Human Soil Ingestion Bioaccessibility | PBET, pH 1.5 PBET, pH 2.5 OSU IVG | OSU IVG SBET | Not evaluated | Not evaluated |
| Plant accumulation Lettuce | Pore water 0.1 M $Ca(NO_3)_2$ | Pore water Mehlich 3 | Not evaluated | Pore water 0.1 M $Ca(NO_3)_2$ |
| Plant accumulation Ryegrass | Pore water 0.1 M $Ca(NO_3)_2$ | Pore water Mehlich 3 | Not evaluated | Pore water 0.1 M $Ca(NO_3)_2$ |
| Soil Invertebrates | Pore water | Not evaluated | Not evaluated | Pore water |

| | 0.5 M Ca(NO$_3$)$_2$ | | | 0.5 M Ca(NO$_3$)$_2$ |
|---|---|---|---|---|

As part of Objective 3, immediately upon receiving funding for this endeavor, a two-day workshop was held bringing together state regulators, DoD site end users, EPA officials, and scientists familiar with soil metal bioavailability. The workshop focused on past, current, and future research endeavors investigating soil metal bioavailability methodologies and the possible use of *in vitro* bioaccessibility values in human health risk assessment and policy. At the kickoff workshop, the research strategy was discussed among scientists, regulators, USEPA, and end-users to advance the acceptance of *in vitro* methods in human health and ecological risk assessment and policy. We incorporated the comments of the attendees of the workshop in our research. In addition, also as part of Objective 3, most of the technical objectives, methods, results, discussion, conclusions, and recommendations of this study are detailed in Appendices A-F, which were written as stand-alone manuscripts for submission as peer-reviewed publications. Publication in peer-reviewed journals is needed to disseminate and ultimately facilitate the results of this study to site managers. In addition, publication in peer-reviewed literature is crucial to ensuring regulatory and community understanding and acceptance of the scientific results. The publication of the results of this study are proceeding.

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# ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| APS | Advanced Photon Source |
| BAF | Bioaccumulation Factor |
| BARGE | Bioavailability Research Group of Europe |
| CEC | Cation Exchange Capacity |
| DoD | Department of Defense |
| EcoSSLs | Ecological Soil Screening Levels |
| EXAFS | Extended X-Ray Absorption Fine Structure |
| GI | Gastrointestinal |
| HQ | Hazard Quotient |
| ICP-AES | Inductively Coupled Plasma Atomic Emission Spectroscopy |
| IVBA | *In vitro* Bioaccessibility |
| IVG | *In vitro* Gastointestinal |
| MLR | Multiple Linear Regression |
| NAVFAC EXWC | Naval Facilities Engineering and Expeditionary Warfare Center |
| OSU IVG | Ohio State University *In vitro* Gastrointestinal Method |
| PBET | Physiologically Based Extraction Test |
| RBA | Relative Bioaccessibility/Bioavailability |
| RBALP | Relative Bioaccessibility Leaching Procedure |
| RMSE | Root Mean Square Error |
| RR | Ridge Regression |
| SBRC | *Solubility Bioavailability Research Consortium* |
| *SBET* | *Simplified Bioaccessibility Extraction Test* |
| SOP | Standard Operating Procedure |
| SSRL | Stanford Synchrotron Radiation Laboratory |
| TRV | Toxicity Reference Value |
| UEF | Urinary Excretion Fraction |
| XANES | X-ray Absorption Near-Edge Structure |
| XRF | X-ray Fluorescence |
| XAS | X-ray Absorption Spectroscopy |

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# 1.0    INTRODUCTION

## 1.1    BACKGROUND

There are thousands of metal-contaminated sites on DoD lands awaiting remediation and closure. Lead, As, Cr, and Cd are toxic (i.e., capable of producing an unwanted, deleterious effect on an organism) metals of particular concern since these metals often control risk-based remedial decisions for soils at DoD sites [1]. Ingestion of contaminated soil by children is the exposure pathway that generally controls remediation goals [2] [3]. With the exception of Pb-contaminated soils, the risk posed by soil ingestion is currently calculated from the total metal (e.g., as measured by USEPA Method 3050B [4]) concentration and the allowed reference dose (non-carcinogen) or cancer slope factor (carcinogen). Reference doses and cancer slope factors are available for most metals and are typically derived from studies of very soluble metal species. In other words, with the exception of Pb, USEPA's risk assessment guidance implicitly assumes a default relative bioavailability of 100%. For the purposes of this study, "bioavailability" refers to the *in vivo* availability of a contaminant to a biological organism (e.g., a plant, human child, or earthworm), while "bioaccessibility" refers to the amount of a contaminant that can be extracted in an *in vitro* procedure. Ruby et al. [5] provides precise definitions of these and other relevant terms (e.g., relative versus absolute bioavailability, etc.). The toxicity assessment for Pb is unique and is based on a pharmacokinetic model of blood Pb. The default bioavailability assumptions in USEPA's blood-Pb model are 50% for food and water and 30% for soil, thus yielding a relative bioavailability in soil of 60% (30%/50%).

Metals in soil, however, can be relatively insoluble and sometimes require aggressive digestion procedures for complete analytical metal recovery. As a result, reference doses developed from studies using soluble metal species may overstate the risk posed by less soluble metals in soils. The generally low bioavailability of Pb and As in mining areas has been well documented. Numerous studies, for example, have shown that Pb in soil [6, 7], mining waste [8, 9] and aggregate [10, 11] is much less bioavailable than more soluble Pb species such as Pb oxide, nitrate, or acetate commonly used in toxicological studies. As a result, Pb in mining environments often exhibits limited bioavailability, and children in Pb mining communities often have lower blood Pb levels than in other areas of the country [12]. Relatively low Pb bioavailability is a consequence of Pb speciation and the corresponding solubility constraints [13] and of kinetically-controlled dissolution due to limited residence times in the gastrointestinal (GI) tract [14]. Risk assessments based on data from studies using soluble metal salts overestimate the risk posed by these soils [15]. In mining-impacted areas, low soil-metal bioavailability is most likely due to the presence of residual low-solubility metal.

Recent SERDP research on certain DOE and DoD hazardous waste and firing range contaminated soils found that nearly all soil-bound Pb was bioaccessible (measured as an *in vitro* surrogate for oral bioavailability). These data were in agreement with highly labile Pb in Pb-spiked soils from around the country that suggested Pb bioaccessibility remained high despite the fact that it was thoroughly adsorbed to various mineral constituents in the soils [16]. Molecular speciation analyses using x-ray absorption spectroscopy (XAS) suggested that Pb(II) was weakly associated with the soil via electrostatic interactions. Apparently in these systems, weak surface bonds between Pb and soil are easily disrupted by the acidic conditions encountered in the stomach. This makes Pb much more bioavailable relative to Pb in mining soils where it most

likely exists as sparingly soluble PbS(s).  However, not all DoD soils have highly bioaccessible Pb, as molecular speciation (e.g., metallic or precipitated as sparingly soluble species) can significantly reduce Pb bioaccessibility (Fendorf, Stanford University, unpublished data).

The reference dose for As is based on human epidemiological studies of As in drinking water. However, soluble As in drinking water is much more bioavailable than insoluble As in soils, the latter being primarily excreted through the feces without absorption in the GI tract [17]. Estimates of risk due to ingestion of As-contaminated soils from some areas will be overstated unless the lower bioavailability of As in these soils is considered [18].  Rodriguez et al. [19] found that the *in vivo* relative bioavailability of As in soils from various mining and smelter sites ranged from 3 to 43%.  They further found that a physiologically-based *in vitro* bioaccessibility method correlated extremely well with the *in vivo* method that used immature swine as a model for the gastrointestinal function of children.

Recent SERDP research has also shown that reference dose criteria used for soil As and Cr is often highly conservative because the indigenous metal-sequestering properties of many soils can significantly lower the bioavailability of ingested toxic metals relative to commonly used default values [16, 20-22].  Our previous results, for example, have shown that numerous DoD soils throughout the U.S. can effectively sequester As(III/V) and Cr(III/VI), significantly decreasing metal bioavailability.  Certain soil physical and chemical properties (e.g., Fe-oxide content, organic matter content, and pH) were highly correlated with decreased metal bioaccessibility, and statistical models were formulated to estimate metal bioaccessibility.  We also used high-resolution spectroscopic techniques, such as XAS, to characterize the chemical environment and speciation of sequestered metals and to verify the modeling results.  Studies conducted at DOE's Stanford Synchrotron Radiation Laboratory confirmed that numerous DoD soils contain natural soil constituents that could reduce mobile Cr(VI) to the less toxic Cr(III) species and oxidize highly mobile As(III) to the less mobile As(V) species.  These redox transformations significantly decreased toxic metal bioaccessibility.  Nevertheless, certain soil conditions were also found to enhance bioavailability of these metals.  For example, when the soil Fe-oxide content for a particular DoD soil fell below 0.5% on a mass basis, the bioaccessibility of As increased dramatically, particularly for alkaline soils [16, 20].  Likewise, for DoD soils low in organic and inorganic carbon, the bioaccessibility of Cr(III) and Cr(VI) is significantly higher relative to soils that possessed these mineral constituents [21, 22].

Unlike Pb and As, most studies of Zn, Cu, Cd, and Ni bioavailability in soils have focused on ecological bioavailability, primarily plant uptake.  It is unlikely that a soil extraction method will replicate the amount of metal absorbed by plants.  The plant uptake system is too complex and dynamic to simulate by simple extraction methods in the laboratory.  A more reasonable approach may be to use soil extraction methods that are based upon soil chemistry and root physiology and that correlate well with plant uptake of metals.  The discipline of Soil Science has used this very concept successfully for the last 50+ years.  Chemical extractants cannot extract plant nutrients in the same manner as a living plant under the conditions of the plant root environment.  However, good correlations between soil extracts and plant uptake has allowed soil scientists to use that relationship to make reasonable predictions of plant available nutrients in soil and subsequent fertilizer recommendations. Plant uptake studies have shown that these metals are largely immobilized by soils, and only a small fraction is bioavailable. Banjoko et al.

[3] found that most of the zinc (78%) present in soil existed in the recalcitrant residual fraction and was not available to maize grown in the soils. When Zn was added to the soil, the Ca-exchangeable fraction decreased to zero within a few days, reflecting the increasing strength of the metal-soil bond over time. Pierzynski [23] found that uptake of Zn by soybeans correlated not with total soil Zn but with more readily available fractions. Similarly, only a readily available fraction of Cu, Cd, and Ni [24-27] is typically bioavailable in soils. In addition, when metal-scavenging Mn [28] or Fe [29] oxyhydroxides are added to soil, metal bioavailability decreases.

Recent SERDP research in our group, using a physiologically-based *in vitro* bioaccessibility method to simulate the human GI tract, has shown that DoD soil-bound metals such as Pb and Cd sometimes remain highly bioaccessible even though they are sequestered by the soil solid phase. Although these toxic metals were effectively bound to the surfaces of mineral constituents in the soil, their weak surface bonds were easily disrupted by the acidic conditions encountered in the simulated stomach environment, allowing them to be much more bioaccessible. These findings are consistent with several bioavailability studies documented by the National Environmental Policy Institute [30] that confirm soils decrease the bioaccessibility of Cd but not nearly to the extent as is observed for metals such as As and Cr. Schroder et al. [31] reported a mean bioaccessible Cd of 63.0% using an *in vitro* gastrointestinal method and mean Cd relative bioavailability of 63.4% in contaminated soils from dosing trials using immature swine. Based on these findings, measurements of key soil properties could be used as indicators to determine whether site remediation is necessary or if more definitive site-specific *in vivo* metal bioavailability studies are warranted. However, site-specific use of bioavailability estimates from soil properties is impeded by the lack of regulatory acceptance. This is rational due to the lack of site-specific investigations that couple *in vivo* bioavailability and *in vitro* bioaccessibility studies with soil properties and microscopic interrogation of the solid phase metals. Several studies have shown good correlations between the *in vitro* Physiologically Based Extraction Test (PBET) or *In vitro* Gastrointestinal (IVG) methods and *in vivo* swine feeding studies for soil Pb [32], soil As [19], and soil Cd [31]. However, none were specifically designed to investigate DoD site-specific soils or considered the role of soil properties in controlling metal bioavailability.

On DoD sites where human exposure is not the main cleanup driver and an ecological risk assessment (ERA) is required, metal bioavailability must be estimated by methods other than PBET or IVG extractions in order to assess exposure for wildlife, soil invertebrates, and plants. Although these extraction techniques may serve to estimate dietary metal exposure in mammalian wildlife, they would not suffice for exposure estimates for soil invertebrates and plants. Similar to human exposure estimates, bioavailability is not currently considered in ecological risk assessments and exposure dose is measured as total metal levels. Instead of references doses, toxicity reference values (TRVs) and ecological soil screening levels (EcoSSLs) have been developed by the USEPA for screening soil metal levels for wildlife, soil invertebrates, and plants. These values have been developed considering soils in which metals are maximally bioavailable. However, site-specific bioavailability adjustments are possible if site metal levels are found to exceed these screening values. A number of techniques are available for making bioavailability adjustments for metals exposure to soil invertebrates and plants. Weak salt extractions (e.g., $Ca(NO_3)_2$ or $CaCl_2$) offer a reasonable alternative to total metal levels and

are currently being employed as an additional method for estimating the bioaccessible fraction of metals in soils.

## 1.2     OBJECTIVE OF THE DEMONSTRATION

The technical objectives of the investigation are:

(1) To provide validation that the relationships between soil properties and *in vitro* bioaccessibility methods can serve as a screening tool for estimating *in vivo* toxic metal bioavailability in DoD soils;

(2) To provide DoD with a scientifically and technically sound method for estimating human and ecological risk associated with metal contaminated soils in place of or as justification for more-detailed, site-specific bioavailability (e.g., animal dosing), and

(3) to promote the use of *in vitro* methods in human health and ecological risk assessments through the upfront involvement of end-users and regulators and the subsequent dissemination of the results of the study in peer-reviewed journals.

## 1.3     REGULATORY DRIVERS

Several recently published studies have summarized the current regulatory climate in regards to these issues.  For example, Ehlers and Luthy [33] summarized the results of the recent NRC report "Bioavailability of Contaminants in Soils and Sediments."  There is neither a national policy nor legal recognition of incorporating bioavailability considerations in site cleanup, although individual states have allowed bioavailability adjustments on a case-by-case basis [5]. To help fill this void, the USEPA is developing guidance and hosted an expert panel discussion in April 2003 on metal bioavailability in soils. Several factors must be aligned at a site to make bioavailability of a contaminant an important consideration: 1) the contaminant whose bioavailability is being investigated is the risk driver; 2) default assumptions of 100% bioavailability are unrealistic; and 3) substantial quantities of contaminated soil and sediment are involved.  Bioavailability arguments should also only be used where site conditions (e.g., land usage, biogeochemical environment, etc.) are unlikely to change over the relevant timeframe. The report advocates long-term monitoring of contaminant sequestration.  A range of tools is available to study bioavailability, from microscopy, to chemical extractions, to bioassays.  Tools that promote mechanistic understanding and lead to the development of a predictive capability are preferred over empirical approaches.  Although the report provides a nice ranking of tools, no single tool achieves the highest ranking in all categories.  The report thus advocates a "weight-of-evidence" approach to tool selection.  The default assumption is typically 100% contaminant bioavailability, which is usually a conservative assumption, because most toxicity tests intentionally use forms of chemicals that are readily absorbed.  Bioavailability assessments can be used to help better prioritize site cleanup.  Most previous assessments have usually come from industry-funded studies at specific sites.

Studies have also focused on the application of these techniques specifically to DoD sites [34, 35].  Except for Pb, the USEPA's human health risk assessment guidance implicitly assumes a default relative bioavailability of 100%.  Bioavailability data can be incorporated into risk assessments at the screening level (Tier IB) as well as in the baseline risk assessment (Tier II).

The results of the Tier IB assessment can be used to remove sites from further consideration or for early identification as to whether or not a bioavailability adjustment is potentially useful in the baseline risk assessment. Bioavailability adjustments should be considered in the following situations: a) a risk estimate slightly or moderately exceeds an acceptable level and triggers required remediation; b) risk-based cleanup goals require extensive remediation; c) remediation is not technically feasible; and d) remediation will adversely impact the environment. If more than three chemicals are risk drivers at a given site, the chances that bioavailability adjustments of a few would significantly affect the required cleanup levels are lessened. Factors that significantly affect whether or not a bioavailability study should be considered include: a) whether the studies can be completed within the required timeframe; b) the cost of the bioavailability study relative to cleanup; c) whether or not existing data support the likelihood of reduced bioavailability.

## 2.0    TECHNOLOGY

### 2.1    TECHNOLOGY DESCRIPTION

The purpose of this demonstration was to demonstrate the ability of soil chemical and bioassay methods to predict metal bioavailability for human and ecological risk assessment. The project sought to provide validated evidence that *in vitro* bioaccessibility methods can serve as time- and cost-effective predictive indices of toxic metal bioavailability in DoD soils relative to *in vivo* uptake studies. By quantifying the extent to which soil properties control metal bioavailability, we have shown that the models developed in our previous SERDP projects can be used with reasonable confidence to predict site-specific metal bioavailability for DoD soils throughout the United States. By coupling *in vitro* and *in vivo* methods at numerous DoD field scale facilities with upfront regulator and end user input, our goal is to facilitate regulatory acceptance of *in vitro* methods and predictive tools for assessing toxic metal bioavailability in contaminated DoD soils as it relates to human and ecological risk.

Soil properties, total metal content, speciation, and bioaccessibility and bioavailability (as measured by various *in vitro* and *in vivo* methods, respectively) were determined for metal contaminated soils collected from three DoD sites for the human health models. A similar approach was taken for the *in vitro* ecological model, which was made more robust by considering an additional eight DoD soils (total of eleven contaminated and eleven control soils for the ecological models).

**Human Health** Metal bioaccessibility and metal bioavailability for three study soils was calculated using soil property-driven models developed from our earlier SERDP studies. Calculated bioaccessibility values were compared with measured bioaccessibility values using *in vitro* gastrointestinal methods for study soils. The physiologically based extraction test (PBET) developed by Ruby et al. [5], was utilized at a variety of pH conditions to estimate metal bioaccessibility for a variety of stomach environments indicative of food intake, or lack thereof. Using the method of Stewart et al. [21, 22], additional soil property-driven models were constructed using the PBET method at these pH values. This is particularly important for Pb contaminated soils since Pb bioaccessibility decreases with an increase in pH [20, 36]. In contrast, As(V) bioaccessibility was minimally influenced by changing pH environments. In addition to PBET, the OSU-IVG [37] method was used to measure bioaccessible As. The ability of the OSU-IVG method to predict contaminant bioavailability was determined.

**Ecological** For ecological risk estimates, metal bioavailability was estimated from multiple regression models developed using bioaccumulation data from 26 soils (CU-1210 and the USEPA-NCEA study [38-43]. Also, the ability of soil extraction methods to predict phytoavailable metals were investigated. Additionally, eight selected DoD sites were tested in addition to the three soils used in the swine study. This was necessary to enhance the robustness of the ecological model [38-43] as has already been done for the human-based model in CU-1166. In the ecological investigations, metal concentrations from *in vitro* DoD soil metal extractions or DoD soil chemical and physical properties were used to predict metal bioavailability to plants and soil invertebrates. Initially, statistical relationships developed for metal availability from a set of twenty-six soils were used to estimate the chemical availability of

metals in DoD soils, based upon total metal levels and soil physical/chemical characteristics. This was followed by extraction of the DoD soils using several soil extraction methods using pore water, dilute calcium nitrate solution, and Mehlich 3 solution. The ability of soil chemical extractants to predict metal bioavailability to plants was determined. Plant and soil invertebrate bioassays were conducted with DoD soils to determine actual toxicity and bioaccumulation, and these results were compared to the model predictions of toxicity and bioaccumulation.

## 2.2 TECHNOLOGY DEVELOPMENT

**Human Health** Within SERDP CU-1166, a predictive model, the Soil BioAccessibility Tool (SBAT) [44] was developed to assess the relative bioavailability of toxic metals in soils. The model was built on the premise that key soil physical and chemical properties (e.g., Fe-oxide content, organic matter content, pH) were statistically correlated with metal bioaccessibility (as measured by *in vitro*, PBET technique). Model results were found to be in good agreement with molecular level metal speciation studies and *in vivo* swine feeding studies [20, 36]. Nevertheless, model validation using *in vivo* studies on actual DoD field samples was lacking. Such an endeavor is critical if the model is ever to obtain end-user and regulatory acceptance.

In addition, recent publications within our group, investigating the bioavailability of As in soil have found that an *in vitro* bioaccessibility method correlated extremely well with the *in vivo* method that used non-DoD soils and immature swine as a model for the gastrointestinal function of children [19]. Similar findings have been reported for soil bound Pb and Cd where the *in vitro* PBET method correlated very well with *in vivo* swine feeding studies [31, 32]. The Ohio State University IVG (OSU-IVG) method has been shown to be correlated with As [37], Pb [45], and Cd [31]. Our research team members also belong to the Bioavailability Research Group of Europe (BARGE) where we have established an international collaboration that seeks to demonstrate the appropriateness of *in vitro* methods for assessing risk associated with soil metal bioavailability. The UK and several countries within the EU have used our (United States) data of coupled *in vitro* and *in vivo* soil metal bioavailability to convince the regulatory community, in their respective countries, that *in vitro* measurements of soil metal bioaccessibility are acceptable estimates of *in vivo* soil metal bioavailability, at least at mining sites. However, although site-specific bioavailability adjustments have been made at some sites, regulators in the United States remain uncertain that the *in vitro* methods alone can adequately predict soil metal bioavailability in humans.

**Ecological** Prior ecotoxicological studies within our group have also been completed that show soil properties similarly affect the bioavailability of As, Cd, Pb, and Zn for soil invertebrates and plants . Measures of metal exposure based upon soil extraction techniques, such as dilute salts [42, 43, 46, 47], have been coupled with soil chemical and physical properties to develop statistical relationships for estimating metal bioavailability for soil organisms. These statistical models are the first step in the development of models capable of predicting the toxicity of metals to soil invertebrates and plants.

Based on our previous scientific and technical advances in the area of *in vitro* and *in vivo* metal bioavailability in soils, we believed that it was timely to apply these techniques to DoD site-specific problems. Such an effort would validate bioaccessibility and bioavailability estimates based on *in vitro* methods and soil properties for DoD sites. Close cooperation with regulators

and end users would lead us closer to regulatory acceptance of *in vitro* methods for assessing toxic metal bioavailability in soils and use of the validated predictive tool SBAT.

Our team has also been involved in research addressing the ecological risk of metals in soil systems. Basta, Dayton and Lanno conducted soil ecotoxicological research for a USEPA-NCEA research project "An Integrated Soil Chemical and Toxicological Approach for the Development of Ecological Screening Levels for Heavy Metals in Soil" (NCEA-ORD Award # CR 827230-01-0) that involved developing methods for determining metal exposure in soil to earthworms and plants using chemical analysis methods other than total metals. Experiments were conducted in twenty-two soils differing in physical/chemical characteristics to develop statistical models relating soil characteristics to bioavailable levels of metals and toxicity in plants and earthworms. This project was followed by CU-1210 (Determining the Bioavailability, Toxicity, and Bioaccumulation of Organic Chemicals and Metals for the Development of Ecological Soil Screening Levels) that examined in greater detail the factors affecting the bioavailability, bioaccumulation, and toxicity of As, Cd, Pb, and Zn to soil invertebrates and plants. The results of our research have also lead to studies examining the physiological partitioning of metals in soil invertebrates and collaborations with researchers at RIVM (Bilthoven, The Netherlands) and the Vrije Univeristeit (Amsterdam, The Netherlands).

## 2.3    ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

The goal of this initiative was to provide field-validated evidence that *in vitro* bioaccessibility methods can serve as predictive indices of toxic metal bioavailability in DoD soils relative to the more costly and time intensive *in vivo* uptake studies.  By quantifying the extent that soil properties control metal bioavailability, we have shown that the predictive models developed in our earlier SERDP studies can be used with a reasonable level of confidence to predict site-specific metal bioavailability for DoD soils throughout the United States.  We believe that this upfront investment by ESTCP to compare *in vitro* methods with *in vivo* investigations can potentially save DoD significant remedial cost in the long term.

The lack of wide-spread regulatory acceptance of the *in vitro* methods is the largest potential limitation to widespread application.  Another potential limitation with using this technology at DoD sites is that there are different types of metal-contaminated sites within the DoD, e.g., small arms firing ranges, paint residues, past pesticide use, and manufacturing/maintenance activities. The bioavailability of a given metal could vary widely between sites, underscoring the ultimate need for site-specific adjustments.

# 3.0 PERFORMANCE OBJECTIVES

One of the performance objectives was to test the bioavailability screening tools developed in our earlier SERDP studies, which correlate chemical speciation, bioaccessibility, bioavailability, and toxicity of metals (Pb, As, Cd, Cr) in DoD soils as measured by biological models used to evaluate ecological risk (e.g., plants, earthworms) and human risk (e.g., immature swine model) (Table 3-1). Since ingestion is often the primary human risk driver at contaminated sites [1], human risk by ingestion was evaluated rather than dermal pathways. Only three sites were considered for the *in vivo* swine dosing studies due to the experimental cost. The use of *in vitro* ecological models were further verified by comparison with *in vivo* ecological bioassay studies of eleven DoD soils (eleven contaminated, eleven control). At the kickoff workshop, the research strategy was discussed among scientists, regulators, USEPA, and end-users to advance the acceptance of *in vitro* methods in human health and ecological risk assessment and policy.

An important component of the technical approach is to validate and demonstrate the ability of soil property models [20-22, 36] and *in vitro* techniques to predict metal bioavailability and risk (i.e., ecological, human). Results obtained from methods developed for assessing metal risk-based endpoints for human our earlier SERDP studies were compared with results from well-established standard methods used to determine human risk (USEPA Risk Assessment Guidance for Superfund) and ecological risk (USEPA Ecological Risk Assessment).

The agreement between the measured and the model-predicted bioavailability was quantified with the root mean square error (RMSE)

$$\text{RMSE} = \left[ \frac{1}{n_d - n_p} \sum_{i=1}^{n_d} \left( B_i - \hat{B}_i \right)^2 \right]^{1/2}$$

where $n_d$ is the numbers of data points, $n_p$ is the number of adjustable parameters (zero when used in a purely predictive manner as in this project), i is an index, and $B_i$ and $\hat{B}_i$ are the *i*-th measured and predicted bioavailability, respectively. The RMSE, the square root of the mean squared difference between measured and predicted values, is a measure of the average error between the predicted and measured values. Our goal was for our models to produce RMSE $\leq$ 25%.

Overall performance objectives are shown in Table 3.1. A discussion of these performance objectives as well as supporting performance objectives can be found in Appendices A-F.

**Table 3-1. Performance Objectives**

| Performance Objective | Data Requirements | Success Criteria | Results |
|---|---|---|---|
| **Quantitative Performance Objectives** | | | |
| Ecological Bioassays vs. *in vitro* protocol | Agreement between the measured and empirical model-predicted bioavailability | Significant multiple regression correlation criteria and/or Root Mean Square Error ≤25% | Soil Inverterbrate-Yes[1] Plants-Yes |
| | Toxicity and bioaccumulation consistent with speciation | Predictive ability of model confirmed | Soil invertebrates – Mixed[2] Plants-Yes |
| | Estimated risk | Bioassay Hazard Quotients (HQs) and *in vitro* HQs | Soil Invertebrate-Mixed[3] Plants-Yes |
| Swine bioassays vs. *in vitro* protocol | Agreement between the measured and empirical model-predicted bioavailability | Root Mean Square Error ≤25% | Pb and As-Yes Cr-No |
| | Toxicity and bioaccumulation consistent with speciation | Predictive ability of model confirmed | Pb and As-Yes Cr-No |
| **Qualitative Performance Objectives** | | | |
| Technology Transfer | End-user involvement | Kick-off meeting held and comments of end-users incorporated in research design. | Yes |
| Ecological bioavailability protocol | Protocol is applicable for evaluating Pb, Cd, Cr, As in soil | Validated statistical model | Soil invertebrates – Mixed Plants-Yes |
| | End-user acceptance | Results published in peer-reviewed journals. | Pending |
| Human bioavailability protocol | Protocol is applicable for evaluating Pb, Cr, As in soil | Validated statistical model | Pb and As-Yes Cr-No |
| | End-user acceptance | Results published in peer-reviewed journals. | Pending |

1. Many significant multiple regressions, some acceptable RMSE, not applicable to essential elements, Cu and Zn, that were not at toxic levels and are regulated by the organisms.
2. Speciation did not significantly increase the predictive capacity of bioaccumulation models.
3. Bioaccumulation of metals only, so no HQs; comparison to US EPA EcoSSLs did not reveal trends.

# 4.0    SITE DESCRIPTION

## 4.1    SITE LOCATION AND HISTORY

The following three sites were selected for the swine dosing studies:
   Portsmouth Naval Shipyard
   McClellan Air Force Base
   Deseret Chemical Depot

The following sites were used for the ecological bioavailability and *in vitro* bioaccessibility studies:
   McClellan Air Force Base
   Hill Air Force Base
   Marine Corp Air Station Cherry Point
   Travis Air Force Base
   Portsmouth Naval Shipyard
   Naval Support Activity Mechanicsburg
   Concord Naval Weapons Site
   Naval Complex, Pearl Harbor, HI
   Deseret Chemical Depot
   Former Sugarcane Fields
   Oak Ridge National Laboratory

**Hill Air Force Base** Hill Air Force Base is located in Ogden, UT.  The contaminated area was historically used as sludge drying beds during the treatment of water for potable use.

**Travis Air Force Base** Travis Air Force Base is located in Fairfield, CA.  Soils from a former small arms range that operated from 1957 until 1977 contain elevated concentrations of lead and antimony.

**Marine Corp Air Station, Cherry Point** The Marine Corp Air Station is located in Cherry Point, NC.  Soils from a former incinerator site contain elevated concentrations of chromium.

**Naval Support Activity, Mechanicsburg** The Naval Support Activity is located in Mechanicsburg, PA.  Soil from Site 11, which has functioned as a lead ingot stockpile location from the early 1950s until recent years, is heavily contaminated with lead.

**Portsmouth Naval Shipyard** The Portsmouth Naval Shipyard is located in Kittery, Maine. Soils from Site 6 are impacted by particulate deposition from historical land use as a temporary storage area of a variety of materials, including lead battery cell plates.

**McClellan Air Force Base** McClellan Air Force Base is located in Sacramento, CA.  Soils from a former wastewater treatment lagoon are contaminated with high concentrations of lead, chromium, and cadmium.

**Deseret Chemical Depot** The Deseret Chemical Depot is located in Tooele, UT.  Soils from an area that was contaminated with mine tailings from flooding during the 1930s were selected.

**Concord Naval Weapons Station** The Concord Naval Weapons Site is located in Concord, CA. Soils from a site that contains elevated As from pesticide applications were utilized.

**Former Sugar Cane Fields** Former sugar cane fields located in Hilo on the big island of Hawaii contain high concentrations of As. The use of As-based pesticides during the 1920-1940s is believed to be the source of the contaminant.

**Naval Complex, Pearl Harbor** Soils located at the Pearl City Fuel Annex contain high levels of As and Pb. The source of As at this site is thought to be historic pesticide or rodenticide use.

**Firing Range, Oak Ridge National Laboratory** Soils located on the small arms firing range contain elevated concentrations of lead.

## 4.2    SITE GEOLOGY/HYDROGEOLOGY

The soils types and soil physical and chemical properties are shown in Table 4-1 and 4-2. Please see Appendices A and F for more detailed soil characterization.

**Table 4-1      Test Sites and Soil Types**

| Site Name | Site Location | Soil Type |
|---|---|---|
| Travis AFB | Fairfield, CA | Alfisol |
| McClellan AFB | Sacramento, CA | Alfisol |
| Hill AFB | Ogden, UT | Entisol |
| Portsmouth Naval Shipyard | Kittery, ME | Inceptisol |
| NSA | Mechanicsburg, PA | Ultisol |
| MCAS Cherry Point | Cherry Point, NC | Entisol |
| Deseret Chemical Depot | Tooele, UT | Aridisol |
| Concord Naval Weapons Site | Concord, CA | Vertisol |
| Naval Complex, Pearl Harbor | Honolulu, HI | Mollisol |
| Former Sugar Cane fields | Hilo, HI | Andisol |
| ORNL Firing Range | Oak Ridge, TN | Ultisol |

**Table 4-2.** Select Soil Properties of contaminated soil (C) and reference (i.e. uncontaminated) soil (R).  All soils are < 2 mm fraction.

| | units | Cherry Pt C | Cherry Pt R | Concord C | Concord R | Deseret C | Deseret R | Hill C | Hilo C | Hilo R | McCllelan C | McCllelan R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Soil pH, water | | 5.50 | 7.43 | 6.67 | 6.34 | 9.28 | 7.84 | 7.22 | 5.88 | 4.71 | 4.31 | 6.66 |
| Soil pH, $CaCl_2$ | | 5.01 | 6.96 | 6.15 | 5.89 | 7.49 | 6.91 | 7.08 | 5.74 | 4.73 | 4.32 | 6.08 |
| EC | dS/m | 0.892 | 0.353 | 0.111 | 0.189 | 0.544 | 0.480 | 0.989 | 0.820 | 1.53 | 0.276 | 0.119 |
| Alox | mg/kg | 6061 | 909 | 1522 | 1672 | 786 | 1207 | 1175 | 21344 | 5917 | 2175 | 487 |
| Feox | mg/kg | 7506 | 797 | 3664 | 4519 | 863 | 681 | 956 | 25678 | 7535 | 4805 | 804 |
| Mnox | mg/kg | 32.2 | <25 | 641 | 659 | 313 | 381 | 333 | 484 | 85.7 | <25 | 125 |
| Org C | % | 3.71 | 0.758 | 3.13 | 2.17 | 0.645 | 0.792 | 1.50 | 7.77 | 5.69 | 4.36 | 0.360 |
| Total C | % | 4.54 | 1.94 | 3.04 | 2.13 | 2.32 | 1.52 | 2.66 | 8.44 | 5.50 | 4.66 | 0.42 |
| CEC | $cmol_c/kg$ | 9.14 | 3.94 | 27.9 | 27.7 | 8.37 | 13.4 | 11.0 | 17.1 | 10.1 | 13.4 | 12.0 |
| Sand | % | 79.7 | 80.0 | 18.4 | 19.9 | 36.6 | 27.5 | 52.3 | 61.1 | 72.3 | 25.7 | 59.9 |
| Silt | % | 13.5 | 12.2 | 40.9 | 44.3 | 54.7 | 53.2 | 31.3 | 25.3 | 17.8 | 50.2 | 25.2 |
| Clay | % | 6.8 | 7.8 | 40.7 | 35.8 | 8.7 | 19.3 | 16.4 | 7.8 | 2.6 | 24.1 | 14.9 |

Soil pH (water): pH measured in 1:1 soil:deionized water suspension
Soil pH ($CaCl_2$): pH measured in 1:2 soil: 0.01 M $CaCl_2$ suspension
EC: electrical conductivity measured in 1:1 soil:deionized water suspension
Alox, Feox, Mnox:  reactive oxide fraction measured using acid ammonium oxalate extraction

**Table 4-2 (continued).**

| | | Mechanicsburg | | ORNL | | Pearl City | | Portsmouth | | Travis | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | R | C | R | C | R | C | R | C | **R** |
| Soil pH, water | | 8.04 | 7.46 | 4.1 | 3.81 | 7.34 | 7.65 | 6.2 | 6.2 | 7.04 | 6.02 |
| Soil pH, CaCl$_2$ | | 7.04 | 7.12 | 3.53 | 3.14 | 7.28 | 7.47 | 6.04 | 5.72 | 6.46 | 5.63 |
| EC | dS/m | 0.209 | 0.291 | 0.184 | 0.152 | 0.995 | 0.929 | 0.089 | 0.183 | 0.247 | 0.261 |
| Alox | mg/kg | 1615 | 2050 | 388 | 851 | 3502 | 2046 | 3764 | 4149 | 799 | 885 |
| Feox | mg/kg | 1407 | 2492 | 507 | 798 | 44900 | 1977 | 5758 | 2682 | 3088 | 4569 |
| Mnox | mg/kg | 290 | 944 | 27.4 | <25 | 1014 | 492 | 124 | 70.1 | 405 | 547 |
| Org C | % | 0.640 | 1.22 | 0.326 | 0.222 | 2.34 | 0.29 | 1.64 | 1.44 | 1.09 | 1.32 |
| Total C | % | 4.49 | 1.43 | 0.38 | 0.17 | 3.33 | 2.01 | 2.57 | 1.72 | 1.22 | 1.39 |
| CEC | cmol$_c$/kg | 9.74 | 9.58 | 2.79 | 7.90 | 25.9 | 39.4 | 2.73 | 2.68 | 17.3 | 10.8 |
| Sand | % | 29.9 | 9.90 | 45.7 | 9.0 | 48.7 | 54.7 | 89.0 | 86.5 | 47.6 | 29.9 |
| Silt | % | 36.6 | 50.0 | 36.5 | 33.4 | 29.2 | 26.9 | 8.5 | 9.6 | 26.3 | 44.3 |
| Clay | % | 33.5 | 40.1 | 17.8 | 57.6 | 22.1 | 18.4 | 2.5 | 3.9 | 26.1 | 25.8 |

Soil pH (water): pH measured in 1:1 soil:deionized water suspension
Soil pH (CaCl$_2$): pH measured in 1:2 soil: 0.01 M CaCl$_2$ suspension
EC: electrical conductivity measured in 1:1 soil:deionized water suspension
Alox, Feox, Mnox:  reactive oxide fraction measured using acid ammonium oxalate extraction

**Table 4-3. Select Properties of ESTCP Contaminated soils (C) and Reference (uncontaminated) soils (R). All soils are < 250 μm fraction.**

|  | units | Cherry Pt | | Concord | | Deseret | | Hill | Hilo | | McCllelan | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  | C | R | C | R | C | R | C | C | R | C | R |
| **Alox** | mg/kg | 10897 | 988 | 1746 | 1765 | 747 | 1251 | 1548 | 28692 | none | 3415 | 650 |
| **Feox** | mg/kg | 13216 | 821 | 4207 | 4752 | 1037 | 763 | 1358 | 30671 | none | 6248 | 1482 |
| **Mnox** | mg/kg | 54.3 | <25 | 634 | 621 | 293 | 224 | 413 | 635 | none | <25 | 125 |
| **Org C** | % | 5.94 | 0.97 | 2.59 | 1.79 | 0.48 | 0.73 | 2.02 | 9.42 | none | 4.56 | 0.52 |
| **Total C** | % | 7.71 | 1.62 | 3.18 | 2.11 | 2.00 | 1.33 | 3.31 | 10.6 | none | 4.42 | 0.548 |
| **CBD Fe** | mg/kg | 10824 | --- | 12749 | --- | 6044 | --- | 4530 | 29606 | --- | 6030 | --- |

Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction
CBD Fe: citrate-bicarbonate-dithionite extractable Fe

## 4.3    CONTAMINANT DISTRIBUTION

The contaminant distributions within the soils are shown in Table 4-3.  Please see Appendices A and F for more detailed soil characterization.

**Table 4-4      Metal concentrations in contaminated (C) and reference (R) soils (dry weight basis)**

| Soil | | Cd mg/kg | Pb mg/kg | Cr mg/kg | Ni mg/kg | As mg/kg | Zn mg/kg | Cu mg/kg |
|---|---|---|---|---|---|---|---|---|
| Mechanicsburg | R | <1.0 | 33 | 56 | 36 | 17 | 97 | 19 |
| | C | <1.0 | 120 | 39 | 29 | 15 | 98 | 25 |
| Cherry Point | R | <1.0 | 17 | 13 | 3.5 | 1.7 | 32 | <1.0 |
| | C | 19 | 114 | 876 | 78 | 6.9 | 486 | 167 |
| Travis | R | <1.0 | 17 | 43 | 23 | 8.1 | 70 | 19 |
| | C | <1.0 | 2034 | 42 | 29 | 11 | 225 | 148 |
| Concord | R | <1.0 | 16 | 79 | 98 | 7.8 | 101 | 50 |
| | C | <1.0 | 22 | 77 | 92 | 220 | 112 | 54 |
| McCllelan | R | 0.7 | 15 | 126 | 60 | 6.1 | 32 | 14 |
| | C | 22 | 193 | 699 | 87 | 9.9 | 448 | 241 |
| Point Loma | R | <1.0 | 8.7 | 23 | 6.8 | 3.7 | 61 | 11 |
| Portsmouth | R | <1.0 | 48 | 14 | 8.4 | 10 | 60 | 12 |
| | C | 1.1 | 3069 | 11 | 62 | 11 | 500 | 185 |
| Deseret | R | <1.0 | 20 | 27 | 17 | 11 | 83 | 15 |
| | C | <1.0 | 19 | 24 | 16 | 438 | 85 | 13 |
| ORNL | R | <1.0 | 12 | 48 | 15 | 14 | 85 | 14 |
| | C | <1.0 | 966 | 16 | 4.2 | 5.0 | 30 | 65 |
| Pearl | R | 1.4 | 13 | 233 | 182 | 4.1 | 133 | 110 |
| | C | 3.6 | 1466 | 185 | 196 | 619 | 1804 | 423 |
| Hilo | R | 1.3 | 153 | 120 | 561 | 22 | 282 | 69 |
| | C | 5.9 | 2134 | 140 | 417 | 660 | 1889 | 224 |

**Point Loma soil was uncontaminated.**

# 5.0    TEST DESIGN

## 5.1    CONCEPTUAL EXPERIMENTAL DESIGN

**Soil collection and characterization** A portable field X-ray fluorimeter was used to identify target metal concentrations in the collection areas prior to collecting 10 to 12 buckets of soil, each containing 25 kg. Since the metal concentration in soil can vary greatly between and within the sample buckets, all soil collected from each site was mixed to produce a homogenous composite sample to be used for all investigations.  Although the homogenization procedure described below may have impacted the oxidation state of the target metals, it ensures that the characteristics observed using synchrotron X-ray techniques are the same as those used for *in vitro*, ecological bioaccessibility, and swine-dosing bioavailability tests. The disadvantage is that there may be some differences in soil characteristics compared with the soil in its local environment. These differences are expected to be minimal in that the soil samples were collected from the surface, and therefore already exposed to an oxidizing atmosphere; none of the soils were from wetlands or other reducing environments. The homogenization procedure is not expected to affect distribution of target metals on soil particles, so X-ray fluorescence microprobe mapping provides an accurate record of elemental associations that supports interpretation of the metal distribution on soil particles.

Soils were air dried prior to homogenization in a heavy duty electric powered mixer with a 9 ft$^3$ plastic drum over six hours. A large cement mixer was modified to allow simultaneous homogenization and sieving (<2 mm) of large amounts (250+ kg) of contaminated soil by using a steel cone attachment fitted with a 2-mm sieve. The steel cone attachment, custom built for the cement mixer, allows (i) greatly improved homogenization, (ii) improved safety by greatly reducing exposure to contaminated dust from the project soils, and (iii) improved efficiency and recovery of homogenized soil. The mixer is equipped with a dust trap to avoid air dispersion of the material. For soils where clumping is an issue, hardened ceramic balls were placed in the mixer with the soil in order to enhance aggregate breakup without grinding the soil, which could alter its native particle size distribution. Soils were next sieved to <2 mm with a subsample sieved to <270 um. The <2mm samples were used in the *in vitro* and *in vivo* plant and earthworm model studies whereas the <270 um samples were used in the *in vitro* and *in vivo* swine model studies and for synchrotron X-ray interrogation. To verify that soil samples are homogeneous, numerous subsamples (10 or more) were acid digested using USEPA method 3051a followed by Cr, As, Cd, and Pb analysis. Soils are archived at Ohio State University where *in vitro* and *in vivo* plant and earthworm model investigations were performed.

Select, yet the most pertinent (based on our previous SERDP-funded research), soil chemical and physical properties were quantified using established analytical procedures.  The soil properties were measured on all soils are total metal analysis, total organic and inorganic carbon, amorphous and crystalline Fe-oxide content, Mn-oxide content, particle size analysis (sand, silt, clay content), cation exchange capacity (CEC) and soil pH.  This information was used in the statistical models to assess the influence of soil properties on metal bioavailability as measured by *in vitro* and *in vivo* techniques.

**Metal speciation and chemical environment** In an effort to validate the physical significance of the soil property models used to describe the bioaccessibility of metals in the DoD soils, the

mechanisms of enhanced metal sequestration and solid-phase metal speciation were quantified with a variety of high-resolution surface spectroscopy techniques. X-ray absorption spectra on bulk samples of the <270 µm size fraction were collected at the Stanford Synchrotron Radiation Laboratory (SSRL) in May 2007 (beam line 2-3; Pb and As analysis) and January 2008 (beam line 11-2, Cr analysis). In both cases a Si(220) monochrometer was used to control the energy of the incident beam, calibrated by metal foils or known reference compounds. Data were collected in fluorescence geometry using a 13- or 30-element germanium solid-state detector (BL 2-3 and BL 11-2, respectively). Samples were ground to fine powder and mounted in teflon sample holders sealed with Kapton tape. Between three and 25 scans were collected on each sample.

Data files were imported into the Samview module of the X-ray absorption spectroscopy processing program Sixpack [48] where monochrometer energy calibration was verified or corrected, and individual scans were examined to ensure that each solid-state detector channel had successfully recorded data. Noise recorded in malfunctioning channels was eliminated before averaging scans. The averaged data was then imported into the program Athena [49]. The near-edge portions of the X-ray absorption near-edge structure (XANES) were examined and first derivatives calculated to determine the energy position of the absorption edge. Next, spectral backgrounds were subtracted and the extended fine-structure portions of the spectra (EXAFS) were expressed in K-space ($Å^{-1}$), where K represents the momentum wave-vector. The resulting $\chi(K)$ files were imported into the program Artemis [49] for analysis of the EXAFS.

Least squares fitting algorithms of the EXAFS function were applied to determine nearest and second-nearest neighbor atomic identities, coordination numbers, and distances from the target metal(loid), using theoretical phase and amplitude functions generated by the program FEFF[50]. First-shell coordination environments were identified, informed by the oxidation state information obtained from XANES. The energy offset parameter $E_0$ was constrained to be the same for all atoms included in the fit. Wave amplitudes corresponding to the coordination number around the target metal were allowed to vary, as were the interatomic distances. The Debye Waller factor, a parameter that varies as a function of static and vibrational atomic disorder [51], was held constant and constrained to be the same for all atoms in the first shell.

For samples containing As, theoretical multiple scattering paths within As tetrahedral were generated from the mineral structure of scorodite ($FeAsO_4 \cdot 2H_2O$). Phase and amplitude functions corresponding to 3-leg paths of the form As-O-O-As (12 paths) and 4-leg paths of the form As-O-As-O-As (16 paths) were generated in Artemis using the IFEFFIT module. To test whether including multiple scattering contributions improved the fit for As K edge EXAFS, the multiple scattering paths were applied with distance and degeneracy parameters fixed to their original values, and the Debye Waller factor constrained to 0.001 [52].

Following first-shell fits, second-shell fits were performed if peaks in Fourier transforms of the EXAFS data representing interatomic distances (uncorrected for phase shift) provided evidence of more distal backscatterers. Potential identities of second-shell backscatterers were informed by the soil chemical analyses and, when available, results of the X-ray fluorescence microprobe mapping performed at APS (described below).

Microbeam X-ray techniques were performed at the Advanced Photon Source (Argonne National Laboratories) bending magnet beam line 20-BM, operated by the Pacific Northwest Consortium Collaborative Access Team (PNC-CAT), in February 2008. Microbeam X-ray fluorescence (XRF) spectroscopy was used to assess spatial distributions of the target elements on the soil particle surfaces. Soil grains were dispersed onto Kapton tape, covered with a second layer of tape, and placed at a 45° angle to the incident beam. An initial location on the sample with multiple, well-spread out particles was chosen with the aid of a video camera. A constant focal position for all samples was maintained by moving each sample on a motorized rail until it was in focus by a second camera with a viewer outside the hutch. Two-dimensional fluorescence microprobe maps were then acquired to ascertain the distribution of target elements in relation to soil particles.

The images were processed on-site using the PNC-CAT software 2d Scan Plot version 2. Individual element distributions (in relation to the dead-time corrected incident X-ray intensity), and mapped representations of element ratios, were compared visually to detect the areas highest in the target metals to choose locations for collecting microbeam X-ray absorption spectra. In cases where the metal association with other elements was not uniform, more than one spot was chosen. For preparation of X-ray fluorescence map figures, target elements mapped in 2d Scan Plot were saved as jpeg images. These images were imported into the SMAK image processing software package [53], where intensity was re-plotted on a log scale to better visualize the distribution of elements, and converted to greyscale.

X-ray energy at the beamline was controlled using an $N_2$-cooled Si(111) double-crystal monochrometer. The beam energy was calibrated using an Au foil placed below the beam path and above a caldiode solid-state detector. Part of the beam was deflected downward to excite the foil, and the absorption reading at the caldiode was normalized to the counts in an ion chamber upstream. The beam was focused by means of a 100 mm K-B mirror to approximately 5 μm.

Locations for X-ray absorption spectra (XAS) were chosen from the XRF microbeam maps, described above. At locations where the target metal(loid) appeared elevated on the map, a multichannel analyzer was employed to measure fluorescent X-ray intensity over a range of energies. Elements (atomic number Z>15) present at that location were identified by the energies of the emission peaks. At selected locations, XANES data were collected using a multielement Ge solid-state detector. Each detector element was set up to record the fluorescence intensity within the emission energy range corresponding to a target metal. Twelve detector elements were utilized for each of the contaminants (Cr, As, Pb), and their signals were summed to obtain the relevant XANES spectrum. The summed data was processed using the software Athena, as described above for the spectra collected at SSRL.

These data provided an improved conceptual understanding of the molecular-level speciation of Pb, Cd, Cr, and As in the soils, and how the molecular speciation influenced the resulting bioaccessibility. The metal speciation results were used to confirmed macroscopic observations of metal bioavailability for both the *in vitro* and *in vivo* methods.

More specifically, the geometric relationship between a metal and its nearest neighboring atoms were interpreted to indicate whether it was adsorbed onto a mineral surface or part of the internal

mineral structure. This was accomplished by evaluating the identities, distances, and coordination numbers to atoms closely neighboring the metal by comparison of the EXAFS with theoretical phase and amplitude functions generated from postulated coordination chemistry scenarios.

A metal that is structurally incorporated into the mineral structure likely will not become bioavailable unless the mineral decomposes, whereas a metal that is adsorbed to a particle surface may be mobilized into the dissolved phase if chemical conditions change. For example, introduction of competing ions that can displace the adsorbed metal, a pH change, or a change in redox conditions can destabilize the metal-particle association. An outer-sphere association (electrostatic attraction) is generally less stable than an inner-sphere association (direct chemical bond).

Both As and Cr exhibit multiple potential oxidation states that influence their toxicity. Dissolved As(III) is typically more toxic than As(V) and also has a lower affinity with mineral surfaces. For Cr, it is the oxidized form (Cr(VI)) that is more mobile and toxic than Cr(III). The oxidation states were easily distinguished from the XANES by the energy at which radiation was absorbed by an inner-shell electron. The absorption edge shifts to higher energy for oxidized species, and a characteristic pre-edge peak is associated with Cr(VI) [54]. The edge position and shape was also compared with that of mineral reference standards.

### *In vitro* **investigations to assess human health risks**
OSU IVG:  Ohio State University *In-vitro* Gastrointestinal Method The OSU-IVG (described in more detail in Appendix E) is a rapid, inexpensive and reliable screening tool for determining the potential bioavailability (i.e., bioaccessibility) of soil contaminants including As [37].  The OSU IVG method simulates important parameters of the human GI tract under fasting conditions.  The amount of contaminant extracted by the OSU-IVG is assumed to be available for absorption across the intestinal membrane (i.e., bioaccessible) and incorporation into systemic circulation. Contaminant bioaccessibility is expressed as a percentage of the total contaminant content of the 3test sample.  Two bioaccessibility values are determined by the OSU IVG: gastric and insestinal.  For gastric bioaccessibility, 150 mL of gastric solution (0.10 M ACS grade NaCl and 1% porcine pepsin, Sigma Aldrich, St. Louis, MO, Cat. No. P7000) is heated in an open extraction vessel, in a 37º C hot water bath.  When the solution reaches 37º C, the pH is adjusted to 1.8 ± 0.1 using 6 M trace metal grade HCl followed by addition of the soil (1 g, < 250 µm). The sample is thoroughly mixed with the solution to maintain a homogenous suspension.  The pH is continuously monitored and adjusted to 1.8 ± 0.1 for 1 h.  After 1 h, 10 mL of gastric solution is removed for analysis.  The extract is immediately centrifuged (11,160 g for 15 min) and then filtered (0.45 µm).  Filtered extracts are refrigerated (4º C) for preservation prior to analysis.  Intestinal bioaccessibility is determined from the gastric sample.  The gastric sample is adjusted to 6.5 ± 0.1 using dropwise additions of a saturated NaOH solution followed by the addition of 0.563 g of porcine bile extract (Cat. No. B8631) and 0.563 g of porcine pancreatin (Cat. No. P1750 Sigma Aldrich, St. Louis, MO).  The pH is continuously monitored and adjusted to 6.5 ± 0.1.  After 2 h of mixing, 10 mL of intestinal solution is collected for analysis.  The extract is immediately centrifuged (11,160 g for 15 min) and then filtered (0.45 µm).  Filtered extracts are refrigerated (4º C) for preservation prior to analysis.  Three replicates analyses of soil test samples are performed to determine bioaccessible contaminants by OSU IVG.  Extracts

are analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES) or Hg-ICP-AES. Calibration standards, check standards, and dilutions are prepared in 0.1 M ACS grade NaCl, and 0.5 M trace metal grade HCl matrix. A blank and a laboratory control sample are included with each batch of *in vitro* sample extractions for quality control.

PBET – Physiologically Based Extraction Test The physiologically based extraction test (PBET) developed by Ruby et al. [5, 32] was utilized at a variety of pH conditions to estimate metal bioaccessibility for a variety of stomach environments indicative of food intake, or lack thereof. Using the method of Stewart et al. [21, 22] additional soil property-driven models were constructed using the PBET method at these pH values. This is particularly important for Pb contaminated soils since Pb bioaccessibility decreases with an increase in pH [20, 36]. In contrast, As(V) bioaccessibility was minimally influenced by changing pH environments. Triplicate samples of 0.3 g dry soil are placed in 50 mL polyethylene tubes to which 30 mL 0.4 M glycine at pH 1.5 and 2.5 are added. The slurries are quickly placed in a rotating water bath of $37^0$C and agitated at $30 \pm 2$ rpm for 1 hr. After 1 hour the samples are rapidly cooled in an ice bath. Supernatant is separated from the solid via centrifugation. The pH of the supernatant is measured to ensure that the final pH is within $\pm 0.5$ pH units of the initial pH.

Metal bioaccessibility and metal bioavailability for the three study soils was calculated using soil property-driven models developed from our earlier studies. Calculated bioaccessibility values were compared with measured bioaccessibility values using *in vitro* gastrointestinal methods for study soils.

### *In vitro* investigations to assess ecological risks
Soil extraction methods For ecological risk estimates, metal bioavailability was estimated from multiple regression and path analysis models developed using toxicity and bioaccumulation data from 26 soils (CU-1210; previous USEPA-NCEA project). Additionally, 12 selected DoD sites (24 soils) from CU-1166 were tested in addition to the three soils used in the *in vivo* swine test. This was necessary to enhance the robustness of the ecological model from CU-1210 as has already been done for the human-based model in CU-1166. In the ecological investigations, data from *in vitro* DoD soil metal extraction coupled with DoD soil chemical and physical properties were compared to existing statistical relationships for estimating metal bioavailability to plants and soil invertebrates. Initially, statistical relationships developed for metal availability from a set of 26 soils were used to estimate the chemical availability of metals in DoD soils, based upon total metal levels and soil physical/chemical characteristics. This was followed by extraction of the DoD soils using extraction with several soil chemical extraction methods (e.g., pore water, dilute calcium nitrate and Mehlich 3 solution) [46, 47] to actually measure the chemical availability of metals in DoD soils. These measurements were compared to predicted chemical availability estimated by the models to determine the ability of the models to predict metal availability. The statistical models were used to predict the toxicity of the DoD soils to earthworms and plants, assuming additivity of the toxicity of individual metals. Although the various metals in a potential mixture have different modes of toxic action, it is difficult to make any other assumption than additivity of toxicity. However, we attempted to estimate Toxic Units contributed by each metal to get an estimate of potential toxicity. Bioassays were conducted with DoD soils to determine actual toxicity and these results were compared to the model predictions. Comparison of the actual toxicity from bioassays with predicted toxicity from *in vitro* models was used to quantify the ability of *in vitro* models to predict actual ecotoxicity in

field DoD soils. This served as the basis for validation of the *in vitro* methods for field DoD soils.

### *In vivo* **investigations**

Plant Plant bioassays with Perennial ryegrass, *Lolium perrene*; and Lettuce, *Lactuca sativa*, were conducted according to Dayton et al. [38-42] with contaminated soils from DoD to provide plant risk-based endpoints of germination, dry matter growth, and tissue metal concentrations. Metal uptake was monitored in both plant species weekly until a steady state was reached, prior to plant bioassays being performed.

Soil Invertebrate Metal bioavailability and ecotoxicity in contaminated soils collected from DoD sites was assessed using soil invertebrate bioassays with earthworms (*Eisenia fetida*), potworms (*Enchytraeus crypticus*), and collembola (*Folsomia candida*) according to standard protocols [55, 56]. Bioassay endpoints included mortality, reproduction, and internal concentration of metals (bioaccumulation).

Swine Metal bioaccessibility calculated by CU-1166 *in vitro* methods using DoD soils were correlated with metal bioavailability using *in vivo* immature swine dosing trials. The pig has been used as an animal model in a number of research fields including gastroenterology, nutrition, and metabolism. Specific justification for the use of swine in chemical bioavailability studies with soil matrices revolves primarily around biological (anatomical, physiological, biochemical) similarities to humans. There is an extensive database of information on the use of the swine model. Standard operating procedures (SOPs) using the immature swine model developed by Dr. Stan Casteel, University of Missouri-Columbia Veterinary Medical Diagnostic Laboratory, have been approved by the USEPA Region 8 for measuring the bioavailability of Pb from incidental ingestion of soils by children. During the past 10 years, the swine model has served well as a surrogate for study of systemic bioavailability of soil Pb in a sensitive population of humans. More than 30 Superfund Site soils from locations across the nation have been tested. The swine model uses relative bioavailability data as measured by comparing oral absorption of the metal of interest in test soils to oral absorption of some fully soluble form of the metal. The fraction of the absorbed dose of a metal can be measured using concentrations in blood and tissues such as liver, kidney, and bone. For the special case of As, the urinary excretion fraction is most appropriate for estimating relative bioavailability. It has been shown by Weis *et al.* [57] that preliminary site-specific estimates of soil Pb relative bioavailability in 20 soils of concern to the USEPA ranged from 6% to greater than 85%, relative to the absorption measured for Pb from Pb acetate. The model has also been used successfully to assess the bioavailability of Cd and As.

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of Pb from a sample collected from the Portsmouth Naval Shipyard. The test material contained a Pb concentration of 4113 µg/g. The relative bioavailability of Pb in the sample was assessed by comparing the absorption of Pb from the test material to that of a reference material (Pb acetate). Groups of five swine were given oral doses of Pb acetate or test material twice a day for 14 days. The amount of Pb absorbed by each animal was evaluated by measuring the amount of Pb in the blood (measured on days 0, 3, 7, 9, 12, and 15) and the amount of Pb in bone (measured on day 15 at study termination). The amount of Pb present in blood or bone of

animals exposed to test material was compared to that for animals exposed to Pb acetate, and the results were expressed as relative bioavailability (RBA)

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of As from a soil sample taken in the vicinity of the Deseret Chemical Depot.  The soil sample contained an As concentration of 521 ug/g. The relative bioavailability of As was assessed by comparing the absorption of As from the test material to that of a reference material (sodium arsenate). Groups of five swine were given oral doses of sodium arsenate or the test materials twice a day for 14 days; a group of three non-treated swine served as a control.  The amount of As absorbed by each animal was evaluated by measuring the amount of As excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12).  The urinary excretion fraction (UEF) (the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours) was calculated for both the test soil and sodium arsenate using linear regression analysis. The relative bioavailability (RBA) of As in the test soil compared to that in sodium arsenate was calculated as follows:

$$RBA = \frac{UEF\ (test\ soil)}{UEF(sodium\ arsentae)}$$

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of chromium from a soil sample taken in the vicinity of McClellan Air Force Base The soil sample contained a chromium concentration of 593 ug/g. The relative bioavailability of chromium was assessed by comparing the absorption of chromium from the test material to that of a reference material (chromium chloride). Groups of five swine were given oral doses of chromium chloride or the test materials twice a day for 14 days; a group of three non-treated swine served as a control.  The amount of chromium absorbed by each animal was evaluated by measuring the amount of chromium excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12).  The urinary excretion fraction (UEF) (the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours) was calculated for both the test soil and chromium chloride using linear regression analysis. The relative bioavailability (RBA) of chromium in the test soil compared to that in chromium chloride was calculated as follows:

$$RBA = \frac{UEF\ (test\ soil)}{UEF(chromium\ chloride)}$$

Statistics
*The ability of bioaccessibility to predict bioavailability*. Measured bioaccessible Pb and As for DOD test soils was inserted into previously published regression equations used to predict Pb bioavailability [58] and to predict As bioavailability [19, 37, 59].  Predicted bioavailability was compare with the measured 90% confidence interval for Pb and As bioavailability from swine dosing trials.

*The ability of soil properties to predict bioaccessibility*. Measured soil properties for DOD test soils was inserted into previously published regression equations used to predict As bioaccessibility [20, 60] and to predict Cr bioavailability [21, 22].  The root square mean error

for predicted-actual  bioaccessibility values was used to determine the ability of soil properties to predict As ro Cr bioaccessibility.

*The ability of soil properties to predict metal bioavailability to plants.*  Statistical models were developed using soil property and plant uptake data from a combined NCEA and SERDP ER-1210 database.  Both multiple linear regression (MLR) and ridge regression (RR) models were developed.  The developed models were evaluated to determine their ability to predict metal bioavailability to plants for the ESTCP study soils.   Both types of models were fit to the data using PROC REG in SAS 9.2.  For the MLR models, model selection was not performed; we included all five independent variables (pH, OC, FEAL, CEC, and Total) in each model.  For the RR models, an extra penalty term is added to the statistical model.  This penalty term can be tuned to adjust the parameter estimates, increasing the bias in the parameter estimates while decreasing the influence of multicollinearity on the parameter estimates.  These biased estimates produce a model that does not fit the observed data as closely as the MLR.  In all cases, the $R^2$ for the MLR will be superior to the one obtained from the RR.  However, the biased estimates produced by the RR often produce a better predictive model, and that was the central goal of our model development.

When using the RR approach, we chose the value of the tuning parameter by selecting the value that minimizes the PRESS statistic.  The PRESS statistic is calculated by removing each observation, in turn, from the dataset; fitting the model using the remaining n – 1 observations; using the model fit to obtain a predicted value for the removed observation; and calculating the squared error of prediction for the removed observation.  After cycling through each observation in the dataset in this manner, the squared errors of prediction are summed to obtain the final PRESS statistic.  The model with the lowest PRESS statistic is declared to have the best predictive ability.  PRESS statistics cannot be compared between RR models with different dependent variables, and there isn't a specific value of the PRESS statistic that can be considered adequate for declaring a model to have good predictive ability.  However, the PRESS statistic can be used to compare two or more RR models with the same dependent variable.

*The ability of soil extraction methods to predict metal bioavailability to plants*.  Regression models developed using bioaccumulation data from the NCEA study were used to predict contaminant phytoaccumulation in the study soils. Comparison of the actual contaminant phytoaccumulation from bioassays with predicted phytoaccumulation from soil extraction methods were used to quantify the ability of soil extraction models to predict actual phytoaccumulation in field DoD soils.

## 5.2     BASELINE CHARACTERIZATION

Key observations from the synchrotron X-ray studies are (1) Pb is present as adsorbed divalent ions or as organic complexes, rather than in crystalline compounds, in all of the Pb-rich soil samples; (2) Cr is present as Cr(III), the more stable and less toxic of the two common Cr oxidation states, in all three Cr-rich soil samples; and (3) Arsenic is present in the more stable and less toxic form, As(V), in three of the four As-rich soil samples, but is present as both As(III) and As(V) in the sample from the Naval Complex at Pearl Harbor. Arsenic appears to occur as an adsorbed complex on iron oxides in the Concord and Pearl samples, and as an adsorbed complex on aluminum oxides in the Hilo soil sample.  No Pb was found to be bound in

more immobile and less bioaccessible sulfide phases, meaning that most of the Pb-O in the soils can be liberated under acidic conditions (i.e., in the stomach or in the case of percolating acidic soil/groundwater).  The finding that Pb is mobilizable in low pH conditions is supported by previous flow-through and leaching experiments performed on the Cherry Point soils [61]. Please see Appendix A for detailed baseline characterization

## 5.3     TREATABILITY OR LABORATORY STUDY RESULTS

Please see Appendices A through F for detailed study results.
## 5.4      DESIGN AND LAYOUT OF TECHNOLOGY COMPONENTS

There were no technology components deployed in the field.
## 5.5      FIELD TESTING

The nominal project schedule is shown in Table 5-1.  Investigation-derived wastes (IDW) were disposed of onsite at the individual PI's laboratories.  No field equipment was deployed or left in place.
## 5.6      SAMPLING METHODS

Please see Appendices A through F for detailed sampling methods.

## 5.7      SAMPLING RESULTS

Please see Appendices A through F for detailed sampling results.

**Table 5-1     Project Schedule**

| Task | Year 1 | Year 2 | Year 3 |
|---|---|---|---|
| Workshop with regulators, EPA, scientists, end users | ▬▬ | | |
| Prepare State of the Science and Regulatory Acceptance White Paper | ▬▬▬ | ▬ | |
| Prepare site selection memorandum and Draft and final Demonstration Plan | ▬▬▬▬ | ▬▬▬ | |
| Identify sites, collect and characterize soil | ▬▬▬▬ | ▬▬▬ | |
| Quantify *in vitro* bioaccessibility | | | ▬▬ |
| Quantify *in vivo* bioavailability | | ▬ | ▬▬▬ |
| *In vivo* ecological bioassays (plant/invert) | | ▬ | ▬▬▬ |
| *In vivo* swine dosing trials | | ▬▬ | ▬▬ |
| Metal speciation with XAS | | ▬ | ▬ |
| Model validation | | | ▬▬ |

The above schedule was based on a nominal project start-date of July 1, 2005.  Individual PI start dates varied depending on when funding vehicles were in place.

# 6.0 PERFORMANCE ASSESSMENT

The technical objectives of the investigation were: (1) To provide validation that the relationships between soil properties and *in vitro* bioaccessibility methods can serve as a screening tool for estimating *in vivo* toxic metal bioavailability in DoD soils; (2) To provide DoD with a scientifically and technically sound method for estimating human and ecological risk associated with metal contaminated soils in place of or as justification for more-detailed, site-specific bioavailability (e.g., animal dosing), and (3) to promote the use of *in vitro* methods in human health and ecological risk assessments through the upfront involvement of end-users and regulators and the subsequent dissemination of the results of the study in peer-reviewed journals.

Performance Objectives 1 and 2 involved testing the bioavailability screening tools developed in our earlier SERDP studies, which correlate chemical speciation, bioaccessibility, bioavailability, and toxicity of metals (Pb, As, Cd, Cr) in DoD soils as measured by biological models used to evaluate ecological risk (e.g., plants, earthworms) and human risk (e.g., immature swine model). Only three sites were considered for the *in vivo* swine dosing studies due to the experimental cost.  The use of *in vitro* ecological models were further verified by comparison with *in vivo* ecological bioassay studies of eleven DoD soils (eleven contaminated, eleven control).

An important first step was characterizing the molecular-level speciation of the metals in the soil with the use of x-ray absorption spectroscopy.  Synchrotron X-ray fluorescence microprobe mapping, microbeam X-ray absorption spectroscopy, and bulk sample X-ray absorption spectroscopy were used to determine the oxidation state and molecular coordination environment of As, Pb, and Cr in eleven study soils with variable soil properties. *In vivo* swine dosing trials to determine metal bioavailability, *in vitro* gastrointestinal studies to determine metal bioaccessibility, soil extraction procedures and soil properties used to predict metal bioavailability to plant and soil invertebrates and ecological bioassay studies were also performed on the same set of soils.  Findings from synchrotron X-ray studies indicated that Pb is adsorbed as divalent ions or present as organic complexes, rather than in crystalline compounds. Chromium and As are present in their more stable and less toxic inorganic forms, Cr(III) and As(V), except in soil from the Naval Complex at Pearl Harbor, where both As(III) and As(V) are present. Arsenic is bound to iron oxides in the Concord and Pearl samples, and to aluminum oxides in the Hilo soil sample. Arsenic-bearing soils may require more site-specific approaches to remediation. Lead was not bound in sulfide phases that would be considered stable, meaning that most of the Pb-O in the soils may be liberated under acidic conditions (i.e., in the stomach).

Metal bioaccumulation and toxicity to soil invertebrates (*E. andrei, En. crypticus, F. candida*) were examined in ESTCP metal-contaminated soils (with paired reference site soils) comprising a wide range of physical and chemical characteristics and metal levels. The predictive ability of a number of different models relating soil properties to oligochaete metal bioaccumulation and toxicity as a screening tool for estimating metal bioavailability in soils was examined with the intent of validating some of these models for predicting metal bioaccumulation in soil-dwelling oligochaetes.

Key elements for predicting bioaccumulation of metals by soil invertebrates include metal concentration in the soil, soil physicochemical characteristics, and time.  In this study, we examined the application of various models, with varying degrees of success, in predicting the

bioaccumulation of metals by earthworms from ESTCP soils. The models can be divided into three categories: 1) Metals for which a large number of models exist in the literature (e.g., Pb, Cd); 2) Metals for which few models exist in the literature (e.g., Cr, Ni); and, 3) Essential metals (e.g., Cu, Zn).

When applying literature-based metal bioaccumulation models to assess Cd and Pb bioaccumulation by earthworms in metal-contaminated field soils, 98% of the variability in earthworm Cd concentrations could be predicted by a model comprising total soil Cd, organic matter content, and soil pH, while 95% of the variability in earthworm Pb concentrations could be predicted by a model including total soil Pb and soil pH. However, both these models over-predicted metal bioaccumulation (Cd 106%; Pb 272%) so their use in predicting bioaccumulation may be limited. A large portion of the variability in the tissue concentrations of As (90%), Cr (77%), and Ni (88%) could be estimated by their concentrations in soil. Even though just a few bioaccumulation models exist for these metals, the models for As (24.2%) and Cr (13.6%) provided acceptable predictions of metal uptake, while the Ni model severely over-predicted uptake (689%). However, for the essential metals Cu and Zn, total soil concentrations combined with soil properties provided a reasonable prediction of tissue concentrations for Cu (24.7%) but not for Zn (590%). A model relating BAF of Cd to soil properties provided acceptable predictions of Cd BAFs by *En. crypticus* from ESTCP soils (20%) while no relationship was evident between BAFs and observed metal burdens for Pb and Zn.

Models developed relating 0.5 M $Ca(NO_3)_2$-extractable Cd and Pb to earthworm metal residues did not provide a better prediction of Cd and Pb concentrations in earthworms exposed to ESTCP soils than models selected from the literature that predicted earthworm metal concentrations based upon total metal levels and soil physicochemical characteristics. Models incorporating toxicokinetics of metals were only available for Cd and provided reasonable estimates of Cd concentrations in earthworms (19%). These results indicate that there are no models for a specific metal that would provide good predictions of metal bioaccumulation in all soils and situations.

**Table 6-1. Summary of the prediction of metal bioaccumulation by earthworms (*Eisenia fetida*) or potworm (*Enchytraeus cryticus*) using soil property or soil extraction data.**

| Approach | Metal | Model | Summary and ability to predict metal body burdens |
|---|---|---|---|
| Soil properties | As | $\ln As_{ew} = 0.9884 * \ln As_s - 1.747$<br>Sample et al. 1998 | Based on total As levels; $R^2=0.90$; under predicts 0.8-16-fold, most soils 0.8-3.3 fold; RMSE = 24.2% |
| | Cd | $\ln Cd_{ew} = 6.018 + 0.787 * \ln Cd_s - 0.106 *OM - 0.402 * pH$    Ma et al. 1983 | Based on total Cd, organic matter, pH; $R^2=0.98$; over predicts 3.8-11.3-fold; only eight data points above DL; RMSE = 106% |
| | Cr | $\log Cr_{ew} = 0.69 * \log Cr_s - 1.05$<br>Peijnenburg et al. 1999a | Based on total Cr; $R^2=0.73$; under predicts 0.8-7.4-fold; RMSE == 13.6% |
| | Cu | $\log Cu_{ew} = 0.435 * \log Cu_s + 0.39$<br>Morgan and Morgan 1988 | Based on total Cu; $R^2=0.45$; under predicts 1.3-5.2-fold; RMSE = 24.7% |
| | Ni | $\log Ni_{ew} = 0.98 * \log Ni_s + 0.67$<br>Neuhauser et al. 1995 | Based on total Ni; $R^2=0.88$; over predicts 11-95-fold; RMSE = 689% |
| | Pb | $\log Pb_{ew} = 2.65 + 0.897 * \log Pb_s - 3.56 * \log pH$<br>Corp and Morgan 1991 | Based on total Pb and pH; $R^2=0.95$; over predicts 0.5-25-fold; RMSE = 272% |
| | Zn | $\log Zn_{ew} = 1.45 * \log Zn_s + 0.42$<br>Peijnenburg et al. 1999a | Based on total Zn; $R^2=0.62$; under predicts 1.3-5.2-fold; RMSE = 590% |
| | Cd | $C_w = 9.32 * e^{-0.008*28} + Cd_s *0.052/0.008*(1-e^{-0.008*28})$<br>Yu and Lanno 2010 | Based on Cherry Point and McLellan soils where total Cd is same as model concentration, one prediction is the same as observed and one is 2-fold higher; with all 8 data points – RMSE = 19% |
| Calcium Nitrate Extraction | Cd | $\log Cd_{ew} = 0.27 * \log Cd_{Ca(NO3)2} + 2.1$<br>$R^2 = 0.66,$ | Only two soils – Cherry Point, McLellan – with total extractable Cd levels; over predicted earthworm Cd 3-6.8-fold; RMSE = 111% |
| | Pb | $\log Pb_{ew} = 0.32 Pb_{Ca(NO3)2} + 97$<br>$R^2 = 0.39, P=0.008$ | Only five soils with extractable Pb; over predicted 1.1-3.6-fold; RMSE = 161% |
| | Zn | $\log Zn_{ew} = 0.02 Zn_{Ca(NO3)2} + 2.12,$<br>$R^2=0.084, P=0.21$ | Only four soils with extractable Zn; under predicted 1.3-2-fold; RMSE = 101% |

| BAF - Soil properties *En.crypticus* | Cd | log BAF=1.17-0.92*log Clay Peijnenburg et al. 1999b | Only six soils where BAF could be calculated; acceptable under-prediction; RMSE = 21% |
|---|---|---|---|
| | Pb | log BAF=0.35-0.36*pH Peijnenburg et al. 1999b | No relationship |
| | Zn | log BAF =3.47-0.46 *pH-0.67*log $Al_{ox}$ Peijnenburg et al. 1999b | No relationship |

Contaminant phytoaccumulation was also determined from plant bioassays for soils from eleven study sites. For ecological risk estimates, metal phytoavailability was estimated from soil-property driven multiple regression models developed using bioaccumulation data from two previous study studies. A separate approach involved the use of soil extraction methods, used to estimate metal(loid) phytoavailability, to predict contaminant phytoaccumulation. Regression models developed using bioaccumulation data from a previous study sponsored by the National Center for Environmental Assessment were used to predict contaminant phytoaccumulation in the study soils. Comparison of the actual contaminant phytoaccumulation from bioassays with predicted toxicity from *in vitro* models were used to quantify the ability of *in vitro* models to predict actual phytoaccumulation in field DoD soils. This was the basis for validation of the soil property or soil extraction methods for field DoD soils. The predictive capability required by a soil property/soil extraction models depends on the degree of accuracy of contaminant phytoaccumulation determined by the risk assessor. With some exceptions, both methods were able to predict phytoavailability at <35% of the measured contaminant tissue value. In general, soil property models were predictive of tissue As, Cd, and Pb. Exceptions were Deseret for As (ryegrass), Hill for Cd (lettuce), and Portsmouth for Pb. In general, the predictive capability of soil extraction methods was adequate to excellent with the exception of Hill for Cd (lettuce) and Portsmouth for Pb.

The predictive capability of soil property / soil extraction models to predict plant phytoaccumulation is summarized as follows.

**Table 6-2.** Summary of the Prediction of Contaminant Phytoaccumulation using Soil Property or Soil Extraction Soil Data

| Approach | Model or Soil Extraction | Ability to Predict Tissue As | | Ability to Predict Tissue Cd | | Ability to Predict Tissue Pb | |
|---|---|---|---|---|---|---|---|
| | | Lettuce | Ryegrass | Lettuce | Ryegrass | Lettuce | Ryegrass |
| Properties | MLR | 4† Concord Over , 5x‡ | 4 Deseret Over, 80x | 4 Hill Under, 1.7x | 4 | 7 Portsmouth Over, 1.3x ORNL Under, 1.3x | 7 Portsmouth Under, 1.2x |
| | RR | 4 | 4 Deseret Over, 80x | 4 Hill Under, 1.7x | 4 | 7 Portsmouth Over, 2x ORNL Over, 2x | 7 Portsmouth Over, 1.7x |
| Soil Extraction | Pore water | 3 | 3 All sites Over, 2x | 3 | 3 Hill Under, 1.6x | 4 Portsmouth Under, 4x | 4 Portsmouth Under, 3.3x |
| | Mehlich 3 | 4 | 4 all sites Over, 2x to 5x | NA | NA | NA | NA |
| | Calcium Nitrate | NA | NA | 3 Hill Under, 10x | 3 Hill Under, 4x | 4 Portsmouth Under, 2x | 4 Portsmouth Under, 2.5x |

† Number of contaminated soils evaluated.

‡ Over prediction of tissue As concentration by a factor of five

One of the main objectives of the project was to determine the ability of *in vitro* gastrointestinal methods (i.e., bioaccessibility methods) to predict measured contaminant bioavailability in contaminated soils from study sites. Equations used to predict bioavailability from bioaccessibility methods are available for Pb and As.

Relative bioavailable Pb was determined for the Portsmouth soil in our study. The PBET methods (pH 1.5 and 2.5) were able to accurately predict *in vivo* RBA for the Portsmouth soil. The predicted RBA for the PBET method at pH 2.5 was closer to actual *in vivo* RBA than pH 1.5. However both methods predict RBA Pb within the 90% confidence interval. The OSU IVG method IVBA Pb was very close to the *in vivo* RBA Pb. However, information on the ability of the OSU IVG method to predict RBA Pb is very limited whereas in depth validation studies have been conducted for the RBALP (i.e., PBET) method. These results support the use of the PBET method at pH 1.5 and 2.5 to accurately predict *in vivo* RBA Pb. Future validation studies where this approach is expanded from the Portsmouth soil to other DoD soils will increase the confidence of using *in vitro* methods to predict *in vivo* RBA Pb.

**Table 6-3.** Comparison of measured and predicted RBA Pb for the Portsmouth soil

| Measured Pb RBA, % | | Predicted Pb RBA | | | | OSU IVG pH 1.8 |
|---|---|---|---|---|---|---|
| | | PBET pH 1.5 | | PBET pH 2.5 | | |
| Mean | 90 % CI† | IVBA, % | RBA, % | IVBA, % | RBA, % | IVBA, % |
| 99 | 70 - 127 | 83.3 | 86.9 | 80.4 | 106.2 | 102.5 |

† CI = confidence interval

Results from our study show both the OSU IVG and SBRC method were able to predict RBA As in the Desert soil. The predicted RBA As by all methods ranged from 12.2 % to 16.2%, which is comparable to the *in vivo* RBA As of 14%. Further validation studies of these methods for other contaminated soils from different DoD contaminant sources are warranted. A study investigating the relationship between *in vitro* IVBA Cr and *in vivo* RBA Cr has not been reported. Thus, it was not possible to evaluate the ability of bioaccessible Cr to predict *in vivo* RBA Cr. In our study, a novel immature swine dosing model was used to determine the *in vivo* RBA Cr for the McClellan soil. RBA Cr was 107% with a 90% confidence interval ranging from 76% to 169%. *In vitro* IVBA Cr PBET method, used to measure bioaccessible Cr at pH 1.5 and at pH 2.5, was 10.1% and 19.0%, respectively. The *in vitro* IVBA values were much lower than the *in vivo* RBA Cr. Further research is needed before IVBA can be used to predict *in vivo* RBA Cr.

**Table ES-4.** Comparison of measured and predicted RBA As for the Deseret soil

| Measured As RBA, % | Predicted As RBA | | |
|---|---|---|---|
| | OSU IVG gastric | OSU IVG intestinal | SBET gastric |

| Mean | 90 % CI† | IVBA, % | RBA, % | IVBA, % | RBA, % | IVBA, % | RBA, % |
|---|---|---|---|---|---|---|---|
| 14 | 13-15 | 8.45 | 15.0 | 8.47 | 16.2 | 10.6 | 12.2 |

† CI = confidence interval

In general, all of the in vitro methods predicted in vivo RBA As with 90% confidence.

Studies of the determination of soil properties on *in vivo* bioavailability or *in vitro* bioaccessibility are very limited. To our knowledge, these relationships have not been reported for Pb and limited studies exist for As and Cr. Key soil physical and chemical properties (e.g. particle size, CEC, Fe-oxides, TOC/TIC, pH) were identified as controlling the extent of toxic metals bioaccessibility as measured using the PBET that simulated the digestive system of humans. The bioaccessibility results (*in vitro*) were found to be in excellent agreement with molecular-level metal speciation studies, which confirmed that key soil properties control metal bioavailability.

The ability of soil properties to predict As and Cr bioaccessibility (IVBA) was dependent on the contamination source. In general, IVBA As measured by PBET and OSU IVG could be predicted from measured soil Fe properties including Feox or CBD Fe for soils where arsenical pesticide was the contaminant source. However, soil properties of the Deseret soil, where mine tailing was the contaminant source, was not predictive of the measured IVBA As. This finding suggests arsenic may occur as discrete minerals from the mining operation. It is likely the insoluble As minerals in the mining waste did not appreciably dissolve and react with soil components. Therefore, its chemical speciation and IVBA solubility will depend on the mining waste mineral not soil property.

The ability of soil properties (i.e., clay, organic and inorganic carbon) to predict and Cr bioaccessibility (IVBA) was dependent on the contamination source. Good agreement between the measured IVBA Cr and predicted IVBA Cr was found for Hill and McClellan soils. Poor agreement between the measured IVBA Cr and IVBA Cr predicted by soil properties was found for the Cherry Point soil. Differences in Cr chemical speciation in soil may offer an explanation. Water or wastewater treatment was the contaminant source for the Hill and McClellan soils. Incinerator ash was the contaminant source for the Cherry Point soil.

Summary of  Soil Properties to Predict Metal Bioavailability
Soil properties, able to predict metal (bio)availability for several contaminated soils in this study, are summarized in the following table. At a minimum, soil property information needed from a site investigation for all contaminants studied are soil pH, clay content, organic C, inorganic C, reactive Fe and Al  (FEAL, Feox and/or CBD Fe). Other properties not studied that will affect ecological endpoints include soil salinity and the presence of other toxicants.

**Table 6-5.** Summary of Soil Properties to Predict Metal Bioavailability

|  | Contaminant |  |  |  |
|---|---|---|---|---|

|  | Pb | As | Cr | Cd |
|---|---|---|---|---|
| Human Soil Ingestion Bioaccessibility | Not evaluated | Feox and FeCBD | Clay content, total organic C, inorganic C | Not evaluated |
| Plant accumulation Lettuce | pH, OC, FEAL | pH, OC, FEAL | Not evaluated | pH, OC, FEAL |
| Plant accumulation Ryegrass | pH, OC, FEAL | pH, OC, FEAL | Not evaluated | pH, OC, FEAL |
| Soil Invertebrates | pH | Total metal | Total metal | pH, OM |

These properties will **not** predict metal bioavailability for all soils. A major finding of this study is the contaminant source and likely speciation greatly affects the ability of soil property to predict metal bioavailability. Metal bioavailability was not able to be predicted for several soils where the contaminant source was unweathered mining waste or discrete inorganic mineral forms such as coal ash. Soil properties should NOT be used to predict contaminant bioavailability in these soils. More research on contaminant source and speciation is needed to determine when soil properties can provide an accurate assessment of metal bioavailability. Currently research is in progress, including research funded by SERDP (i.e., ER-1742) to determine the relationship between As speciation and ability to predict As bioavailability to humans.

Summary of Soil Extraction Methods to Predict Metal Bioavailability
Soil exaction methods, able to predict metal (bio)availability for several contaminated soils in this study, are summarized in the following table. Both PBET and OSU IVG were able to very accurately predict RBA As and Pb but for only for 1 soil each. The number of soils evaluated were very limited because of cost constraints associated with in vivo dosing trails required to measure contaminant RBA. More research is needed to evaluate the ability of these methods to predict RBA Pb and RBA As on other contaminated soils.

Soil pore water was able to predict plant tissue concentration of Pb, As, and Cd. Soil extraction with 0.1 M Ca(NO$_3$)$_2$ was able to predict cationic metal contaminants(i.e. Pb, Cd) but was not evaluated for anionic As contamination. The ability of simply water or dilute calcium nitrate to predict phytoavailable contaminant suggests high solubility of these contaminants in soils. Thus, it is likely that with 0.1 M Ca(NO$_3$)$_2$ would have also been a good predictor of plant As. However, two cautions should be heeded. The accuracy of these extraction methods to predict plant tissue contamination was limited to $\pm$ 35%. Similarly to metal bioaccessibility results, metal bioavailability was not able to be predicted for several soils where the contaminant source was unweathered mining waste (i.e. Deseret) or discrete inorganic mineral forms such as coal ash (i.e. Cherry Point). Soil extraction methods listed in the summary table should NOT be used to predict contaminant bioavailability in these soils. More research on contaminant source and speciation is needed to determine which soil extraction methods can provide an accurate assessment of metal bioavailability.

**Table 6-6.** Summary of Soil Extraction Methods to Predict Metal Bioavailability

| | Contaminant | | | |
|---|---|---|---|---|
| | Pb | As | Cr | Cd |
| Human Soil Ingestion Bioaccessibility | PBET, pH 1.5 PBET, pH 2.5 OSU IVG | OSU IVG SBET | Not evaluated | Not evaluated |
| Plant accumulation Lettuce | Pore water 0.1 M Ca(NO$_3$)$_2$ | Pore water Mehlich 3 | Not evaluated | Pore water 0.1 M Ca(NO$_3$)$_2$ |
| Plant accumulation Ryegrass | Pore water 0.1 M Ca(NO$_3$)$_2$ | Pore water Mehlich 3 | Not evaluated | Pore water 0.1 M Ca(NO$_3$)$_2$ |
| Soil Invertebrates | Pore water 0.5 M Ca(NO$_3$)$_2$ | Not evaluated | Not evaluated | Pore water 0.5 M Ca(NO$_3$)$_2$ |

As part of Objective 3, immediately upon receiving funding for this endeavor, a two-day workshop was held bringing together state regulators, DoD site end users, EPA officials, and scientists familiar with soil metal bioavailability. The workshop focused on past, current, and future research endeavors investigating soil metal bioavailability methodologies and the possible use of *in vitro* bioaccessibility values in human health risk assessment and policy. At the kickoff workshop, the research strategy was discussed among scientists, regulators, USEPA, and end-users to advance the acceptance of *in vitro* methods in human health and ecological risk assessment and policy. We incorporated the comments of the attendees of the workshop in our research. In addition, also as part of Objective 3, most of the technical objectives, methods, results, discussion, conclusions, and recommendations of this study are detailed in Appendices A-F, which were written as stand-alone manuscripts for submission as peer-reviewed publications. Publication in peer-reviewed journals is needed to disseminate and ultimately facilitate the results of this study by site managers. In addition, publication in peer-reviewed literature is crucial to ensuring regulatory and community understanding and acceptance of the scientific results. The publication of the results of this study are proceeding.

Please see Appendices A through F for a detailed performance assessment.

**7.0 COST ASSESSMENT**

Cost is an important part of the decision making process when doing bioavailability assessments and making risk management decisions. Questions a project manager must ask themselves include:

- How can I balance the cost of in vivo studies with the desire for reduced uncertainty when making risk assessment conclusions?

- What is the potential return on investment of a bioavailability study? Would adjustments to the RBA at the site lead to higher remedial goals? Would higher remedial goals allow for a reduced remedial footprint and reduced costs?

- Is there existing data that indicates reduced bioavailability of metals contaminants at the site?

- Does the project schedule allow for the time required to complete a bioavailability assessment?

The following sections provide cost information to help remediation professionals begin to answer these questions.

**7.1 COST MODEL**

The following tables provide simple cost model information. Site-specific bioavailability assessment will require a sampling and analysis plan, sample collection and reporting. These costs are estimated in Table 7-1. *In vitro* study costs are presented next in table 7-2, followed by costs for the in vivo studies demonstrated in this study.

**Table 7-1. Cost Model for Bioavailability Assessment: Sample Collection and Reporting**

| | **Sample Collection and Reporting** | | | |
|---|---|---|---|---|
| **Cost Element** | **Data Tracked During the Demonstration** | **Unit type: Number** | **Unit Cost** | **Estimated Costs** |
| **Sampling and Analysis Plan** | • Personnel required and associated labor<br>• Materials | Sampling and Analysis Plan Document: 1 | $8,000 | $8,000 |
| **Sample collection and preparation** | • Costs associated with labor and materials tracked | XRF: 1/sample Sample collection: 1/sample Grinding and sieving: 1/sample | $450/sample | $1,350 |
| **Reporting** | • Costs associated with labor tracked | Report documenting results of entire project: 1 | $20,000 | $20,000 |

Assumptions: Approximately 1 acre site with 3 samples. Sample collection and preparation includes necessary grinding and sieving for bioavailability studies.

**Table 7-2. Cost Model for Bioavailability Assessment: *In vitro* Bioaccessibility**

| | **In Vitro Bioaccessibility** | | | |
|---|---|---|---|---|
| **Cost Element** | **Data Tracked During the Demonstration** | **Unit type: Number** | **Unit Cost** | **Estimated Costs** |
| **In Vitro Tests** | • Personnel required and associated labor<br>• Analytical laboratory costs<br>• Reporting | Set of three tests | $600<br><br><br><br>$110 | $1,800<br><br><br><br>$330 |
| **Total** | | | | $2,130 |

Assumptions: Each soil sample includes the following three replicate laboratory tests: reference, contaminated, and lab reference. Approximately 1 acre site with 3 samples. Sample collection and preparation includes necessary grinding and sieving for bioavailability studies.

**Table 7-3. Cost Model for Bioavailability Assessment: Plant Toxicity Tests**

| Plant Toxicity Tests | | | | |
|---|---|---|---|---|
| **Cost Element** | **Data Tracked During the Demonstration** | **Unit type: Number** | **Unit Cost** | **Estimated Costs** |
| **Plant Toxicity Tests –** | • Personnel required and associated labor<br>• Analytical laboratory costs | Lab technician, per unit cost (set of three tests/sample) | $3,000 | $9,000 |
| | | Metals analysis and soil parameters | $500 | $1,500 |
| **Waste disposal** | Hazardous waste or standard soil disposal | | | $200 |
| **Reporting** | | | $55/hr | $275 |
| **Total** | | | | $10,975 |

Assumption: Each soil sample includes the following three toxicity tests: reference, contaminated, and lab reference. Approximately 1 acre site with 3 samples. Sample collection and preparation includes necessary grinding and sieving for bioavailability studies.

**Table 7-4. Cost Model for Bioavailability Assessment: Soil Invertebrate Toxicity Tests**

| | Soil Invertebrate Toxicity Tests | | | |
|---|---|---|---|---|
| **Cost Element** | **Data Tracked During the Demonstration** | **Unit type: Number** | **Unit Cost** | **Estimated Costs** |
| **Soil Invertebrate Toxicity Tests – Earthworm, Potworm, and Collembola** | • Personnel required and associated labor <br> • Analytical laboratory costs | Lab technician, per unit cost (set of three tests) | $4,000 (Earthworm - $1,200 Enchytraeid - $1,200 Collembola - $1,600) | $12,000 |
| | | Metals analysis and soil parameters | $500 | $1,500 |
| **Waste disposal** | Hazardous waste or standard soil disposal | | | $200 |
| **Total** | | | | $4,700 |

Assumption: Each soil sample includes the following three toxicity tests: reference, contaminated, and lab reference. Approximately 1 acre site with 3 samples. Sample collection and preparation includes necessary grinding and sieving for bioavailability studies.


Table 7-5. Cost Model for Bioavailability Assessment: In Vivo Swine Study

| In Vivo Swine Study | | | | |
|---|---|---|---|---|
| **Cost Element** | **Data Tracked During the Demonstration** | **Unit type: Number** | **Unit Cost** | **Estimated Costs** |
| **Soil In Vivo Swine Study** | • Personnel required and associated labor<br>• Analytical laboratory costs | Lab technician, per unit cost | $20,500 | $61,500 |
| | | Animals/Supplies | $7,500 | $22,500 |
| | | Laboratory Analysis | $8,500 | $25,500 |
| **Waste disposal** | Hazardous waste or standard soil disposal | | | $200 |
| **Total** | | | | $109,700 |

All cost elements are provided on a per unit basis in the above tables. It is assumed that for the lower cost options such as an *in vitro* study, more samples could be analyzed leading to a broader understanding of RBA at the site.

## 7.2    COST DRIVERS

A site specific bioavailability analysis will vary in cost according to site specific factors that drive how many and what type of analysis is required. These variations in cost are apparent in the tables shown in Section 7.1. A significant driver in the determination of whether or not to pursue an adjustment of RBA is the potential cost avoidance.

Removal is the primary remedial technology available for soils contaminated with the metals studied. Soil removal, transportation and disposal costs for metal-contaminated soils can exceed $1,000 per cubic yard. A significant reduction in remedial footprint can easily justify the expense of in vivo studies at some sites. An example is provided in Table 7-6 and Figure 7-1. This example shows an Hg-contaminated site where the initial remedial goal of 50 mg/kg was based on the assumption that the Hg at the site was the soluble form $HgCl_2$ and was 100% bioavailable. Speciation and bioavailability studies were done and the risk assessment was revised based on the adjusted RBA of 10%. The final remedial goal for the site was 400 mg/kg reflecting an RBA of 10%, significantly reducing the footprint of the remediation area. The reduced footprint correlated with a more than 100,000 yd$^3$ reduction in soil volume to be removed and avoided almost $50 million in unnecessary remediation costs.

**Table 7-6. Example Bioavailability Adjustment Cost/Benefit Analysis**

| Bioavailability (%) | Remediation Goal (mg Hg/kg soil) | 1000 yd$^3$ excavated | Cost (10$^6$ 1995 $) |
|---|---|---|---|
| 100 | 50 | 120 | 81 |
| 30 | 180 | 54 | 49 |
| 10 | 400 | 10 | 34 |
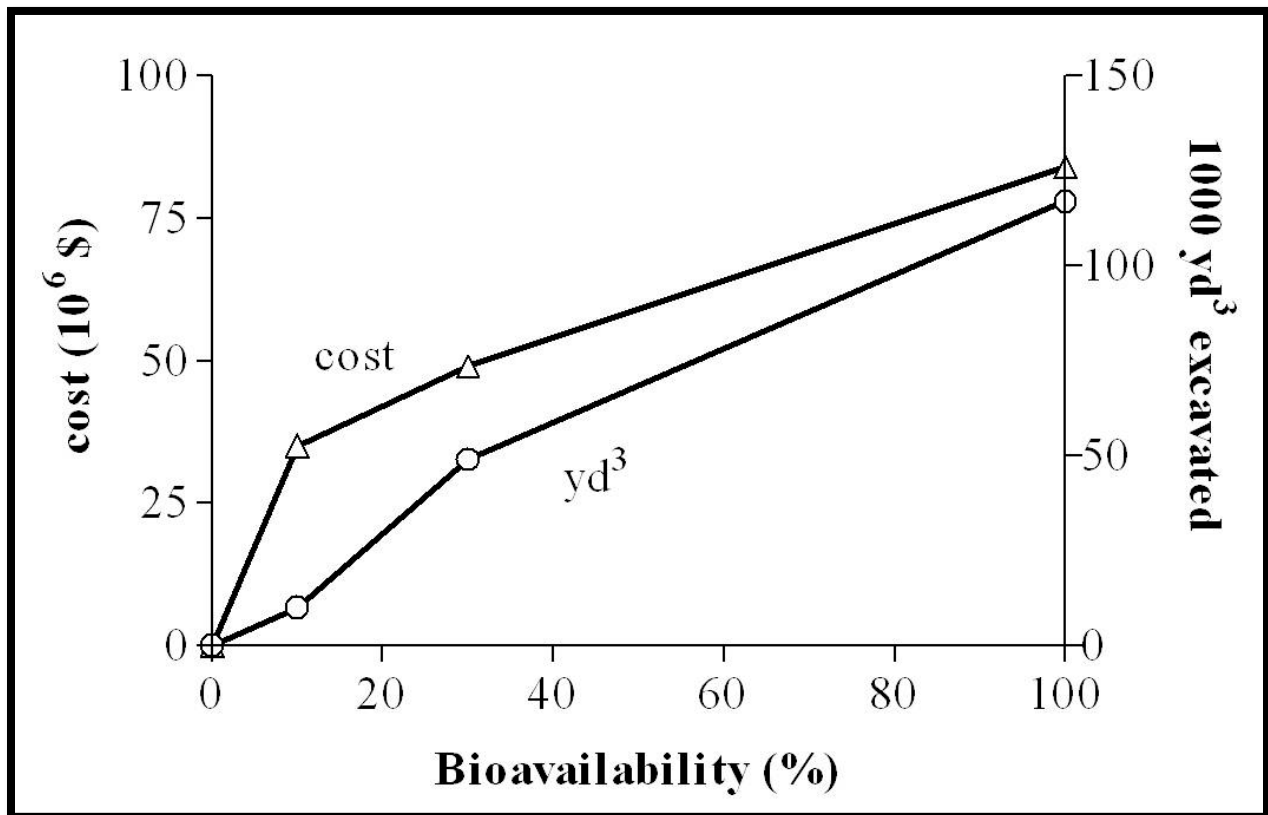


Courtesy of Auburn University

**Figure 7-1. Example Bioavailability Adjustment Cost/Benefit Analysis**

## 7.3    COST ANALYSIS

Consideration of cost should be part of the decision making process when determining whether bioavailability analyses are appropriate for a given site. Figure 7-2 provides a logical process to control costs related to bioavailability analysis. If metals concentrations in site soils indicate unacceptable risk using the hazard quotient (HQ) approach, a review of soil properties and

current bioavailability assumptions should be done. If soil properties indicate that metals may be less bioavailable than assumed in the risk assessment, the next step towards adjusting the RBA is *in vitro* analysis. Before undertaking *in vitro* analysis consideration should be given to the site specific factors impacting the cost/benefit equation for the site. Factors that significantly affect whether or not a bioavailability study should be considered include: a) whether the studies can be completed within the required timeframe; b) the cost of the bioavailability study relative to cleanup; and c) whether or not existing data support the likelihood of reduced bioavailability.

If *in vitro* studies are completed and do indicate reduced RBA, the degree of certainty related to those adjustments should be documented for the project team. Understanding the results of the *in vitro* study in context can help the project team make the decision to use the results of the *in vitro* study in site risk assessment decisions. The team will also have the information necessary to determine if in vivo studies are required for making RBA adjustment decisions at the site and what the potential benefits of such studies are for the site.
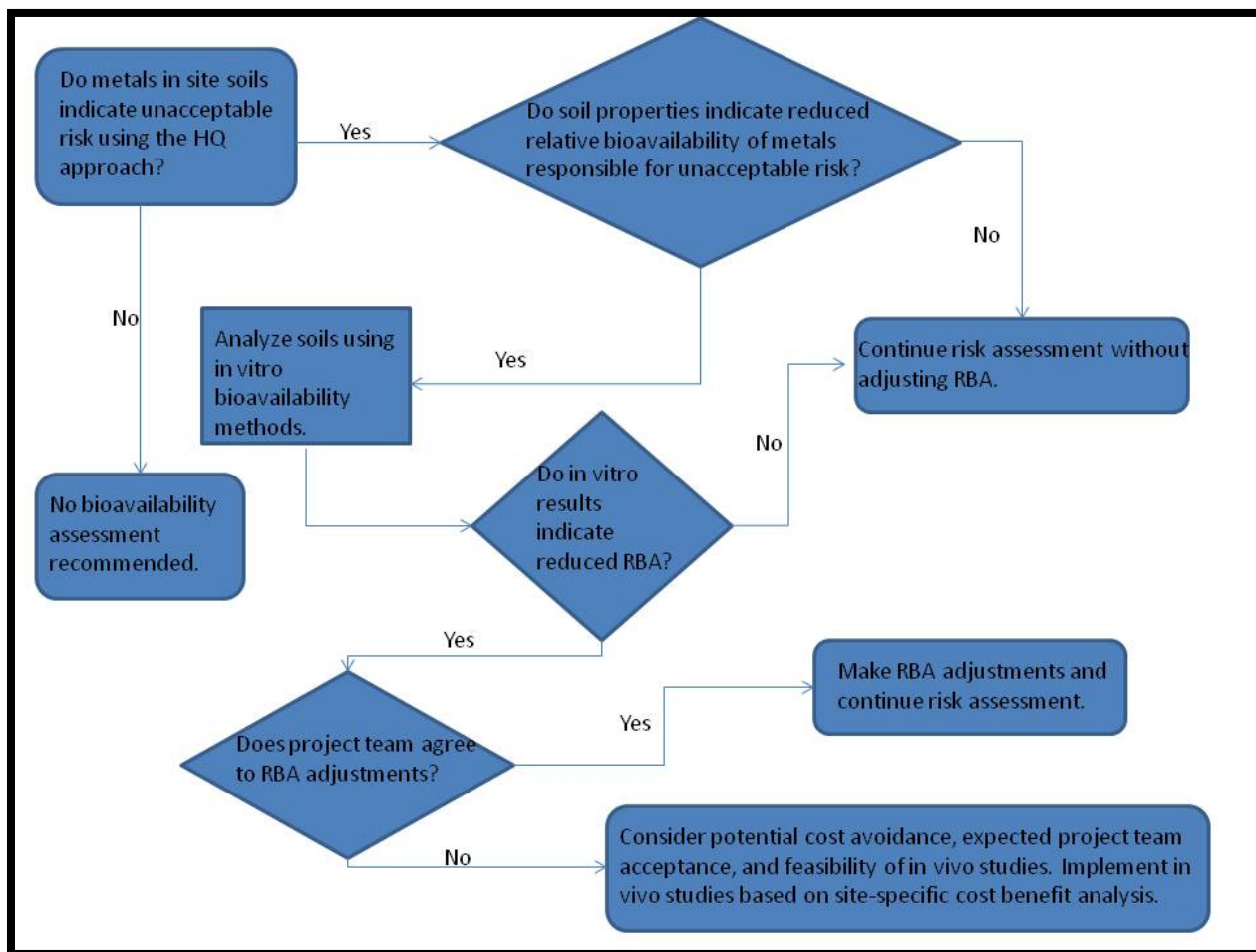


**Figure 7-2. Process to Control Costs Related to Bioavailability Analysis**

## 8.0    IMPLEMENTATION ISSUES

Results from this study show *in vitro* gastrointestinal methods can be used to predict bioavailable Pb and As via soil ingestion human exposure pathway.  However the number of soils / sites were limited due to project costs.  Further validation studies of these methods for other contaminated soils from different contaminant sources are warranted to increase acceptance of these methods in human health risk assessment by regulatory bodies.  The ability of soil properties to predict bioavailability was inconsistent and contaminant source dependent.  Soil properties were accurate predictors for some soil/contaminant source combinations but not others.  Further studies are needed before a more detailed contaminant speciation model can be used to determine which soils may be suitable for estimating metal bioavailability using soil properties.

The predictive capacity afforded by soil property / soil extraction models depends to a large degree on the degree of accuracy of contaminant phytoaccumulation determined by the risk assessor.  With some exceptions, both methods were able to predict phytoavailability at $< 35\%$ of the measured contaminant tissue value.  In general, soil property models were predictive of tissue As, Cd, and Pb.  Exceptions were Deseret for As (ryegrass), Hill for Cd (lettuce), and Portsmouth for Pb.  In general, the predictive capability of soil extraction methods was adequate to excellent with the exception of Hill for Cd (lettuce) and Portsmouth for Pb.

In assessing the bioavailability and toxicity of metals in the soils of this study, it was apparent that soil invertebrates, particularly oligochaetes, exhibited reduced reproduction relative to the laboratory reference soil, in site reference soils. This was most extreme for earthworms, where reproduction in site reference soils was significantly lower in all but one site reference soil. Enchytraeid reproduction was lower in about half the site reference soils, while there was no effect of site reference soil on reproduction in Collembola. This suggests, that of the three soil invertebrates tests, earthworms are the least relevant since the soil types tested were unsuitable for earthworm reproduction regardless of whether elevated levels of metals were present. The reliance on earthworm testing of soils is widespread but may not be correct for certain soils, since *E. andrei* prefer soils rich in organic matter and reproduce poorly in soils with elevated sand or silt content. Enchytraeids are naturally found in a wider array of soils and can thrive in soils with a higher sand or silt content. Arthropods, such as Collembola, are affected even less by soil properties. The evaluation of metal bioavailability in soils with properties not conducive to testing with earthworms should incorporate tests using other soil invertebrates that are either indigenous to the soils being tested or which reproduce adequately in the test soils (e.g., enchytraeids, collembola, mites). In addition, soils found on DoD sites may be composites of soils that have been manually moved from a number of areas and deposited at sites distant from their origin. Additionally, many of these soils may not be suitable for earthworm inhabitation due to physical compaction, low moisture and organic matter content, and the presence of unmeasured chemicals. In short, the soils may be considered test substrates with unique properties, rather than actual soils, and warrant site-specific testing for chemical bioavailability and toxicity rather than assessment using standard extraction and chemical analysis.

Ecological Soil Screening Levels (EcoSSLs) are conservative screening levels for contaminants in soil that are preferentially based upon toxicity data from soils where soil physical and chemical characteristics provide conditions of maximum chemical bioavailability.  At least for

soil invertebrates and plants, there does not appear to be any clear relationship between toxicity and EcoSSL levels for metals in the DoD soils tested, as toxicity was observed in site reference soils as well as those where metal levels did not exceed EcoSSLs. Both field and laboratory research on evaluating the utility of EcoSSL in site specific investigations is warranted.

Regulatory barriers for using bioavailability adjustments in ecological and human health risk assessments are complex and not easily resolvable. Regulatory acceptance of *in vitro* bioavailability in the near term will be on a case-by-case basis with most decisions based on site-specific data. Translating soil properties into field-scale risk assessment adjustments will also require consideration of future site uses that may alter soil characteristics and the subsurface environment and hence, bioavailability. This technical demonstration will contribute to this effort by providing significantly more complete and coupled data sets that link *in vivo* and *in vitro* bioavailability with soil characterization and metal speciation data.

The lack of guidance and policy coupled with time constraints on moving forward with cleanups present a regulatory barrier. The lack of guidance stems from insufficient published data to support the use of bioavailability adjustments in risk assessments. At present, *in vitro* data alone is generally not sufficient to make risk adjustments. More robust data sets are needed that correlate *in vitro* and *in vivo* data. Researchers must collect and publish data in peer-reviewed journals, including information on which *in vitro* tests work and which do not. Keeping regulators and site end-users abreast of these research findings will ultimately pave the way for an enhanced appreciation of *in vitro* methods as tools to estimate metal bioavailability on contaminated DoD sites. The ultimate publication of the results of this study will significantly help bridge this data gap. Publications and abstracts related to this study are described below in Table 8-1.

## PUBLICATIONS

Yu, S., Lanno, R.P. 2010. Uptake kinetics and subcellular compartmentalization of cadmium in acclimated and unacclimated earthworms (Eisenia andrei). Environ. Toxicol. Chem. 29:1568-1574.

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Yu, S., Lanno, RP. 2009. Uptake kinetics and subcellular compartmentalization of cadmium in acclimated and unacclimated earthworms (Eisenia andrei). SETAC 29th Annual Meeting, New Orleans, Louisiana, Nov. 19-23.

Yu, S., Lanno, R.P. 2009. The effect of soil properties on metal bioavailability to earthworms: Validation of laboratory models using metal-contaminated field soils. SETAC 29th Annual Meeting, New Orleans, Louisiana, Nov. 19-23.

Yu, S., Anderson, H., Basta, N, Lanno, R.P. 2008. The effect of soil properties on metal bioavailability to earthworms: Validation of laboratory models using metal-contaminated field soils. SETAC 29th Annual Meeting, Tampa Bay, FL, Nov. 16-20.

**Table 8-1. Publications and Abstracts**

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# APPENDIX A. METAL AND METALLOID SPECIATION IN CONTAMINATED SOILS FROM U.S. MILITARY FACILITIES USING SYNCHROTRON X-RAY TECHNIQUES

Savage, K.S.[1], Covey, A.K.[2], Weinman, B.[3], Lanno, R.P.[4], Casteel, S.W.[5], Basta, N.T.[6], Dayton, E.A.[6], Barnett, M.O.[7], and Hawkins, A[8].

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## ABSTRACT

Cleanup strategies at numerous Department of Defense (DoD) facilities awaiting remediation require a better understanding of how the contaminants of interest are bound in the soils to address their long-term fate and toxicity. Synchrotron X-ray fluorescence microprobe mapping, microbeam X-ray absorption spectroscopy, and bulk sample X-ray absorption spectroscopy were used to determine the oxidation state and molecular coordination environment of arsenic, lead, and chromium in ten soils with variable soil properties, selected from contaminated DoD lands for assessment of health and ecological hazards. *In vivo* bioaccessability studies, swine dosing trials, ecological bioassay studies were also performed on the same set of soils. Findings from synchrotron X-ray studies indicate that Pb is adsorbed as divalent ions or present as organic complexes, rather than in crystalline compounds. Cr and As are present in their more stable and less toxic inorganic forms, Cr(III) and As(V), except in soil from the Naval Complex at Pearl Harbor, where both As(III) and As(V) are present. As is bound to iron oxides in the Concord and Pearl samples, and to aluminum oxides in the Hilo soil sample. Arsenic-bearing soils may require more site-specific approaches to remediation. Pb was not bound in sulfide phases that would be considered stable, meaning that most of the Pb-O in the soils may be liberated under acidic conditions (i.e., in the stomach or in the case of percolating acidic soil/groundwater).

## INTRODUCTION

Numerous sites on Department of Defense (DoD) lands will require remediation. These sites currently rank somewhere on the order of 8000, with most locations owing their contamination to industrial, commercial, training, and weapons testing activities (Figure A-1). With a majority (~70%) of these sites involving soils contaminated with elevated concentrations of various metalloids (As) and metals (Cr, Cd, Cu), and with the DoD charged with the task of remediating these properties for revitalization and development (Salatas et al., 2004), characterizing the extent to which the elevated concentrations pose a human health and/or ecological problem is an important part of determining the best remediation strategies. Successful measures in the management and restoration ~100,000 tons of contaminated soils from landfill and naval shipyard sites impacting the Chesapeake Bay watershed (Lane et al., 2007) set a good precedent for the DoD's ability to effectively deal with the task of cleaning

these properties, but questions still remain about the extent to which the contaminations actually pose a problem.

Currently, models used for risk assessment by the DoD and EPA are predicated on the assumption that the entire pool of contaminants are bioavailable (Stewart et al., 2003a). While elevated concentrations of metals such as arsenic (used as a former pesticide), chromium (used in plating), and lead (used in former firing ranges) are contaminants in DoD soils, little is known about how the contaminants are actually sequestered, as well as the extent to which they actually pose a health problem: contaminants stably sorbed and/or precipitated in crystalline compounds are typically less available, and hence less toxic, than contaminants sequestered in more labile pools of the soils. Since the soils at each of 8000 sites have different bulk geochemical (e.g. pH, organic matter content, sesquioxides potentially providing sorption sites) and physical properties (i.e., specific surface area, surface charge, porosity), it is unclear how toxic and mobile the contaminants are in the different soil materials (Stewart et al., 2003b). For instance, soils in the arsenic-contaminated Concord, Hilo, and Pearl City locations range from matrices comprised of aridisols, andisols, and mollisols, respectively (ESTCP-ER-0517 2007). The different minerals and organic components comprising the soils in each of these locations (i.e., aridisols < mollisol < andisol % organic carbon) could mean that the geochemical and biological availability of As, Cr, and Pb is site-specific, requiring different remediation strategies (Stewart et al., 2003; Chorover et al., 2004; Salatas et al., 2004). The occurrence of Blackfoot's disease in Taiwan is an example of how differences in bulk material can impact toxicity, with naturally occurring organic matter in the Taiwanese sediments resulting in more toxic forms of arsenic poisoning versus other Asian aquifers with high arsenic and low organic content (Reza et al., 2007). Hence, to evaluate the true context within which a contaminant is toxic, it is not only important to quantify how much of a contaminant is present in the soil, it is equally important to characterize the material with which it is associated, and how it is sequestered in that material.

The redox sensitivity of As and Cr adds another level of complexity to determining the level of toxicity. For instance, As(III) is more toxic than As(V) (Cullen and Reimer, 1989), and Cr(VI) is anionic and more toxic than the cationic Cr(III) (Stewart et al., 2003). While Pb is also redox sensitive, it almost always speciates as Pb(II) at Earth surface conditions (Lollar et al., 2004). Despite the high Pb levels associated with some mine sites, Pb is typically not a problem when high levels of sulfide are also present. Under the vadose type of conditions presumably

present in most of the DoD soils, sulfides are not likely to precipitate at high enough levels to effectively sequester all of the Pb, Cr, and As added to the soil. To determine how each of these contaminants is predominantly situated in each of these settings, X-ray absorption spectroscopy can be used on bulk soil samples to see how the As, Cr, and Pb is bound in the soils. Currently, there is no consensus on how these contaminants are interacting with their host soils, making it difficult to determine how available or how immobile they are in their present conditions.

In this study, synchrotron X-ray techniques including X-ray fluorescence microprobe mapping, microbeam X-ray absorption spectroscopy, and bulk X-ray absorption spectroscopy (XAS) were employed to assess the mechanisms of metal sequestration. The XAS near-edge structure, XANES, was utilized to determine the oxidation state of the target metals and the extended fine structure, EXAFS, was employed to assess their atomic coordination environment in the soil samples. Comparison with theoretical models and with spectra from relevant model compounds enable distinction between adsorption and substitution/coprecipitation modes of metal sequestration. The approach was to first identify particular soil grains within the samples that are elevated in the target elements using X-ray fluorescence, followed by microbeam XAS on the targeted regions. Microbeam techniques provide a complementary tool to bulk EXAFS analysis by providing direct information about heterogeneity within the sample.

The geometric relationship between a metal and its nearest neighboring atoms may be interpreted to indicate whether it is adsorbed onto a mineral surface or part of the internal mineral structure. A metal that is structurally incorporated into the mineral structure likely will not become bioavailable unless the mineral decomposes, whereas a metal that is adsorbed to a particle surface may be mobilized into the dissolved phase if chemical conditions change. For example, introduction of competing ions that can displace the adsorbed metal, a pH change, or a change in redox conditions can destabilize the metal-particle association. An outer-sphere association (electrostatic attraction) is generally less stable than an inner-sphere association (direct chemical bond). The type of association can be evaluated geometrically according to the distances between the metal and second-neighbor heavy atoms at the mineral surface.

## MATERIALS AND METHODS
### SOIL SELECTION

DoD facilities with different soil properties, but all contaminated with Cr, As, and/or Pb were selected for in vivo bioaccessability studies, swine dosing trials, ecological bioassay studies, and X-ray interrogation. Soil types hypothesized to strongly sequester metals as well as soil types thought to have poor metal sequestering potential were desired. For example, sandy, high pH aridisols at Hill Air Force Base and Deseret Chemical Depot, with limited capacity to sequester metals, were expected to have high metal bioaccessibility. Silty, neutral pH soils from Travis Air Force Base, with good to excellent metal sequestering properties, were expected to have low metal bioaccessibility. Acidic, Fe-oxide rich soils such as have excellent capacity to sequester anions such as As, and potentially poor capacity to sequester cations such as Cd and Pb. Characteristics of soils chosen for bioaccessibility and X-ray studies are shown in Table A-1.

**SOIL COLLECTION**

A portable field X-ray fluorimeter was used to identify target metal concentrations in the collection areas prior to collecting 10 to 12 buckets of soil, each containing 25 kg. Since the metal concentration in soil can vary greatly between and within the sample buckets, all soil collected from each site was mixed to produce a homogenous composite sample to be used for all investigations. Although the homogenization procedure described below may have impacted the oxidation state of the target metals, it ensures that the characteristics observed using synchrotron X-ray techniques are the same as those used for in vitro, ecological bioaccessibility, and swine-dosing bioavailability tests. The disadvantage is that there may be some differences in soil characteristics compared with the soil in its local environment. These differences are expected to be minimal in that the soil samples were collected from the surface, and therefore already exposed to an oxidizing atmosphere; none of the soils were from wetlands or other reducing environments. The homogenization procedure is not expected to affect distribution of target metals on soil particles, so X-ray fluorescence microprobe mapping provides an accurate record of elemental associations that supports interpretation of the metal distribution on soil particles.

Soils were air dried prior to homogenization in a heavy duty electric powered mixer with a 9 cu ft. plastic drum over six hours. A large cement mixer was modified to allow simultaneous homogenization and sieving (<2 mm) of large amounts (250+ kg) of contaminated soil by using a steel cone attachment fitted with a 2-mm sieve. The steel cone attachment, custom built for the

Table A-1. Soils selected for studies

| Soil | Soil Type | Contaminant | Source of Contamination | pH | Organic Carbon (%) | Oxalate Extractable Fe (g/kg) | Aqua Regia Digestible Contaminant (mg/kg) < 250 um soil sample | Study Use[A] |
|---|---|---|---|---|---|---|---|---|
| Cherry Point | Entisol | Cr, Pb | Incinerator Site | 6.39 | - | 5.33 | 639, 91 | w, iv, s |
| Concord | Vertisol | As | Pesticides | 6.67 | 3.58 | 4.09 | 220[B] | w, iv |
| Deseret | Aridisol | As | Flooding of Mine Tailings | 9.28 | 1.13 | 1.01 | 544 | s |
| Hill | Entisol | Cr | Sludge Drying Beds | 7.22 | 2.4 | 1.32 | 369 | w, iv |
| Hilo | Andisol | As | Arsenic Pesticides | - | - | - | - | s |
| McClellan | Alfisol | Cr, Pb | Wastewater Treatment Lagoon | 4.31 | 6.08 | 6.09 | 574, 164 | w, iv, s |
| Mechanicburg | Ultisol | Pb | Lead Ingot Stockpile | 8.04 | 2.12 | 1.95 | 223 | w, iv |
| ORNL | Ultisol | Pb | Small Arms Firing Range | - | - | - | - | |
| Pearl | Mollisol | As | Pesticides/Rodenticides | - | - | - | - | w, iv |
| Portsmouth | Inceptisol | Pb | Temporary Storage Area (e.g. Lead Battery Cell Plates) | 6.2 | 3.24 | 8.93 | 4113 | w, iv |
| Travis | Alfisol | Pb | Small Arms Firing Range | 7.04 | 2.12 | 3.53 | 2416 | w, iv |

[A] w: ecological bioavailability (worm toxicity tests). iv: in vitro bioaccessibility. s: swine dosing
[B] <2mm soil sample used for aqua regia digestion

cement mixer, allows (i) greatly improved homogenization, (ii) improved safety by greatly reducing exposure to contaminated dust from the project soils, and (iii) improved efficiency and recovery of homogenized soil. The mixer is equipped with a dust trap to avoid air dispersion of the material. For soils where clumping is an issue, hardened ceramic balls were placed in the mixer with the soil in order to enhance aggregate breakup without grinding the soil, which could alter its native particle size distribution. Soils were next sieved to < 2 mm with a subsample sieved to < 270 um. The < 2mm samples were used in the in vitro and in vivo plant and earthworm model studies whereas the < 270 um samples were used in the in vitro and in vivo swine model studies, and for synchrotron X-ray interrogation. To verify that soil samples are homogeneous, numerous subsamples (10 or more) were acid digested using USEPA method 3051a followed by Cr, As, Cd, and Pb analysis. Soils are archived at Ohio State University where in vitro and in vivo plant and earthworm model investigations were performed.

## X-RAY ABSORPTION SPECTROSCOPY

X-ray absorption spectra on bulk samples of the <270 μm size fraction were collected at the Stanford Synchrotron Radiation Laboratory (SSRL) in May 2007 (beam line 2-3; Pb and As analysis) and January 2008 (beam line 11-2, Cr analysis). In both cases a Si(220) monochrometer was used to control the energy of the incident beam, calibrated by metal foils or known reference compounds. Data were collected in fluorescence geometry using a 13- or 30-element germanium solid-state detector (BL 2-3 and BL 11-2, respectively). Samples were ground to fine powder and mounted in teflon sample holders sealed with Kapton tape. Between three and 25 scans were collected on each sample.

Data files were imported into the Samview module of the X-ray absorption spectroscopy processing program Sixpack (Webb, 2004) where monochrometer energy calibration was verified or corrected, and individual scans were examined to ensure that each solid-state detector channel had successfully recorded data. Noise recorded in malfunctioning channels was eliminated before averaging scans. The averaged data was then imported into the program Athena (Ravel and Newville, 2005). The near-edge portions of the spectra (XANES) were examined and first derivatives calculated to determine the energy position of the absorption edge. Next, spectral backgrounds were subtracted and the extended fine-structure portions of the spectra (EXAFS) were expressed in K-space ($\text{Å}^{-1}$), where K represents the momentum wave-

vector. The resulting $\chi(K)$ files were imported into the program Artemis (Ravel and Newville, 2005) for analysis of the EXAFS.

Least squares fitting algorithms of the EXAFS function were applied to determine nearest and second-nearest neighbor atomic identities, coordination numbers, and distances from the target metal(loid), using theoretical phase and amplitude functions generated by the program FEFF (Ankudinov, 2002). First-shell coordination environments were identified, informed by the oxidation state information obtained from XANES. The energy offset parameter $E_0$ was constrained to be the same for all atoms included in the fit. Wave amplitudes corresponding to the coordination number around the target metal were allowed to vary, as were the interatomic distances. The Debye Waller factor, a parameter that varies as a function of static and vibrational atomic disorder (O'Day et al., 1994), was held constant and constrained to be the same for all atoms in the first shell.

For samples containing arsenic, theoretical multiple scattering (MS) paths within As tetrahedral were generated from the mineral structure of scorodite  ($FeAsO_4 \cdot 2H_2O$). Phase and amplitude functions corresponding to 3-leg paths of the form As-O-O-As (12 paths) and 4-leg paths of the form As-O-As-O-As (16 paths) were generated in Artemis using the IFEFFIT module. To test whether including multiple scattering contributions improved the fit for As K edge EXAFS, the multiple scattering paths were applied with distance and degeneracy parameters fixed to their original values, and the Debye Waller factor constrained to 0.001 (Beaulieu and Savage, 2005).

Following first-shell fits, second-shell fits were performed if peaks in Fourier transforms of the EXAFS data representing interatomic distances (uncorrected for phase shift) provided evidence of more distal backscatterers. Potential identities of second-shell backscatterers were informed by the soil chemical analyses and, when available, results of the X-ray fluorescence microprobe mapping performed at APS (described below).

**X-RAY FLUORESCENCE MICROPROBE**

Microbeam X-ray techniques were performed at the Advanced Photon Source (Argonne National Laboratories) bending magnet beam line 20-BM, operated by the Pacific Northwest Consortium Collaborative Access Team (PNC-CAT), in February 2008. Microbeam X-ray fluorescence (XRF) spectroscopy was used to assess spatial distributions of the target elements

on the soil particle surfaces. Soil grains were dispersed onto Kapton tape, covered with a second layer of tape, and placed at a 45˚ angle to the incident beam. An initial location on the sample with multiple, well-spread out particles was chosen with the aid of a video camera. A constant focal position for all samples was maintained by moving each sample on a motorized rail until it was in focus by a second camera with a viewer outside the hutch. Two-dimensional fluorescence microprobe maps were then acquired to ascertain the distribution of target elements in relation to soil particles.

The images were processed on-site using the PNC-CAT software 2d Scan Plot version 2. Individual element distributions (in relation to the dead-time corrected incident X-ray intensity), and mapped representations of element ratios, were compared visually to detect the areas highest in the target metals to choose locations for collecting microbeam X-ray absorption spectra. In cases where the metal association with other elements was not uniform, more than one spot was chosen. For preparation of X-ray fluorescence map figures, target elements mapped in 2d Scan Plot were saved as jpeg images. These images were imported into the SMAK image processing software package (Webb, 2006), where intensity was replotted on a log scale to better visualize the distribution of elements, and converted to greyscale.

## MICROBEAM X-RAY ABSORPTION SPECTROSCOPY

X-ray energy at the beamline was controlled using an $N_2$-cooled Si(111) double-crystal monochrometer. The beam energy was calibrated using an Au foil placed below the beam path and above a caldiode solid-state detector. Part of the beam was deflected downward to excite the foil, and the absorption reading at the caldiode was normalized to the counts in an ion chamber upstream. The beam was focused by means of a 100 mm K-B mirror to approximately 5 μm (Antonette et al., 2001).

Locations for X-ray absorption spectra (XAS) were chosen from the XRF microbeam maps, described above. At locations where the target metal(loid) appeared elevated on the map, a multichannel analyzer (MCA) was employed to measure fluorescent X-ray intensity over a range of energies. Elements (atomic number $Z > 15$) present at that location were identified by the energies of the emission peaks. At selected locations, XANES data were collected using a multielement Ge solid-state detector. Each detector element was set up to record the fluorescence intensity within the emission energy range corresponding to a target metal. Twelve detector

elements were utilized for each of the contaminants (Cr, As, Pb) and their signals were summed to obtain the relevant XANES spectrum. The summed data was processed using the software Athena, as described above for the spectra collected at SSRL.

## RESULTS AND DISCUSSION

### PB- AND CR-RICH SOILS

Lead displays a wide range of coordination environments in soils. Nearest-neighbor oxygen atoms in common Pb minerals can be as close as 2.16 Å in plattnerite [$Pb(IV)O_2$] and up to 2.62 Å in anglesite ($PbSO_4$), and the number of coordinating oxygen atoms can vary from 4 to 12. Furthermore, the coordination environment can be highly symmetrical, with identical first-shell Pb-O distances, or distorted, with three or more Pb-O distances. In some minerals, such as pyromorphite, there are two Pb sites, with different coordination environments. Pb can also be directly coordinated with sulfur, as in galena (Pb-S: 2.97 Å). Sorbed onto iron or aluminum oxides, Bargar et al (1997a, 1997b) found Pb-O distances in the range 2.3 to 2.4 Å. In organic matter complexes, Pb-O distances have been reported as 2.3 Å in lignins (Marmiroli, 2005), 2.41 Å in cellulose (Marmiroli, 2005), 2.32 and 2.46 Å in humates at pH 6 and pH 4, respectively (Xia et al., 1997). Lead can be also be coordinated with carbon, as in trimethyl or tetramethyl lead (Pb-C: 2.14 – 2.18 Å, Glidewell, 1990). Direct Pb-Pb associations occur in organic dilead compounds, in the 2.77 – 2.98 Å range (Glidewell, 1990).

Chromium is generally present in contaminated soils as either tetrahedrally coordinated Cr(VI) or octahedrally coordinated Cr(III). Cr(VI) is the more mobile and toxic species, while Cr(III) is typically present in stable minerals such as chromite and can also substitute for Fe(III) in minerals such as magnetite. The presence of Cr(VI) is indicated in Cr K edge XANES spectra by the presence of a significant pre-edge feature associated with 1s to 3d electron transitions in tetrahedrally coordinated Cr(VI) (Peterson et al., 1997). Because backscattering phase and amplitude paths of Cr and Fe at the same distance are not readily distinguishable, it can be difficult to ascertain the identity of second-shell backscatterers that could represent either Cr or Fe.

Results of X-ray investigation for Pb- and Cr-rich samples are presented in Figure A-2 (X-ray fluorescence microprobe maps) and Figure A-3 (X-ray absorption spectra). In all cases, Pb is divalent, coordinated with oxygen, and does not appear to be associated with crystalline
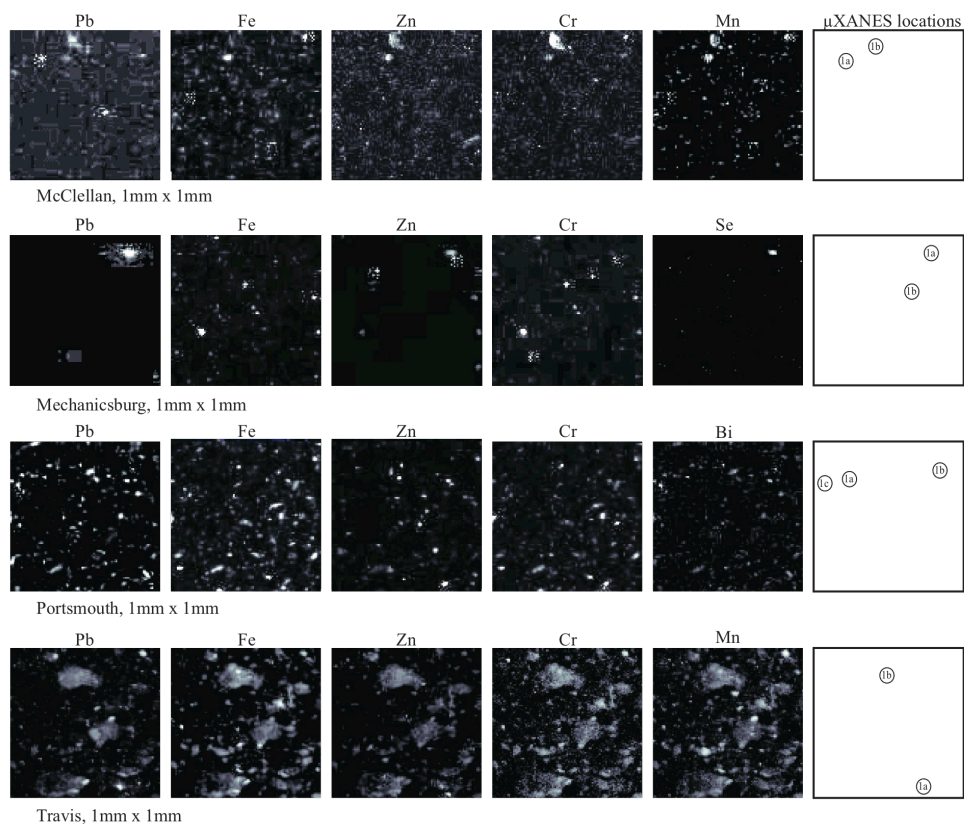
Figure A-2. X-ray fluorescence microprobe maps showing distributions of Pb, Fe, Zn, Cr, and Se, Bi, or Mn in contaminated soil samples collected from the McClellan air force base (Sacramento, CA), Naval Support Activity site (Mechanicsburg, PA), Portsmouth Naval Shipyard (Kittery, ME) and Travis Air Force Base (Fairfield, CA). Locations where microbeam XANES and/or X-ray fluorescence spectra were collected are shown at right.
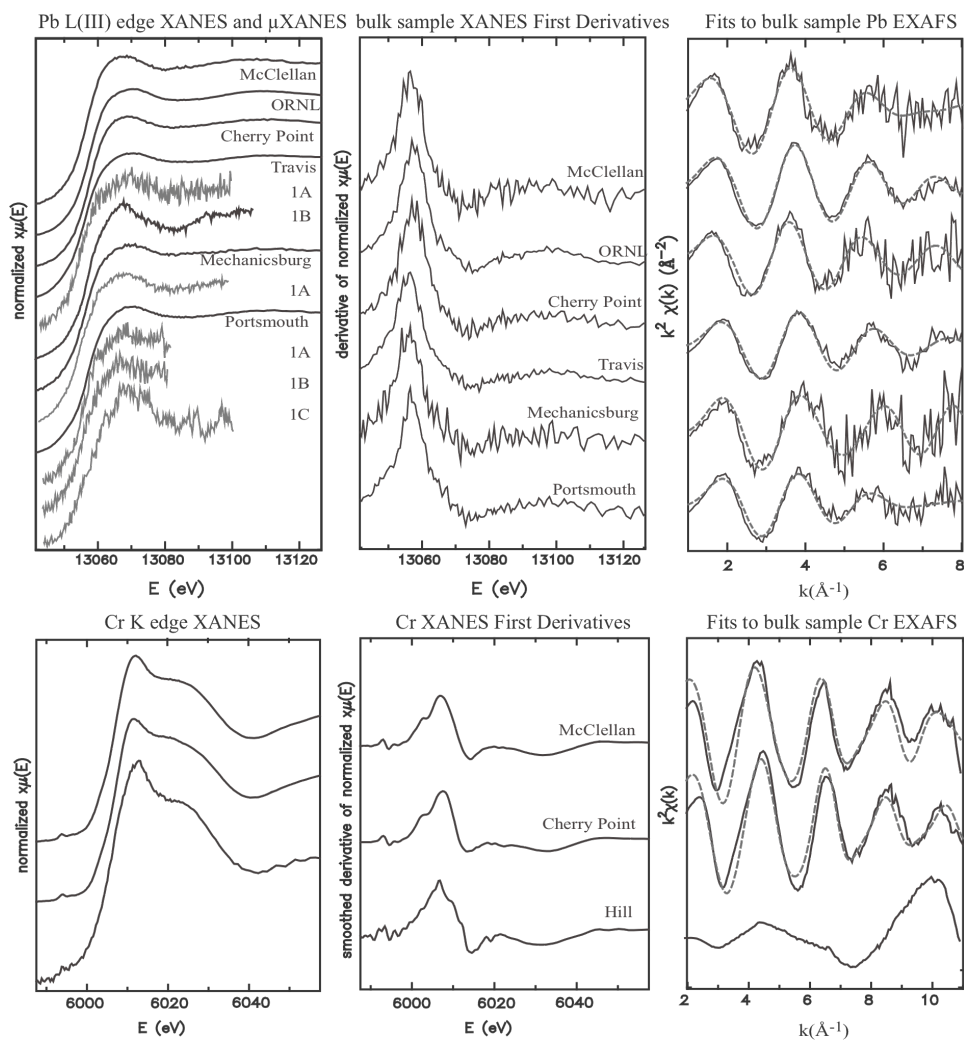
Figure A-3. Lead L(III) edge X-ray absorption spectra and Cr K edge X-ray absorption spectra of contaminated soil samples showing near-edge features for bulk samples and selected microbeam analyses (XANES), first derivatives of XANES spectra for bulk soil samples, and fits to Extended X-ray Absorption Fine Structure spectra (EXAFS) for bulk soil samples. Fit parameters are shown in Table 2.

Table A-2. Parameters used to generate fits to EXAFS data shown in Figures A-3 and A-5.

| Sample | shell | R(Å) | N | s |
|---|---|---|---|---|
| McClellan-Pb | Pb-O | 2.27 | 0.5 | 0.005 |
| | Pb-O | 2.34 | 1.3 | 0.005 |
| | Pb-O | 2.55 | 1.7 | 0.005 |
| | | | | |
| McClellan-Cr | Cr-O | 1.99 | 4.5 | 0.002 |
| | Cr-Cr | 3.04 | 1.0 | 0.0053 |
| | Cr-Fe | 3.39 | 0.4 | 0.0053 |
| | | | | |
| ORNL | Pb-O | 2.25 | 0.6 | 0.005 |
| | Pb-O | 2.36 | 0.8 | 0.005 |
| | Pb-O | 2.48 | 1.6 | 0.005 |
| | | | | |
| Cherry Point-Pb | Pb-O | 2.34 | 1.6 | 0.005 |
| | Pb-O | 2.57 | 0.9 | 0.005 |
| | | | | |
| Cherry Point-Cr | Cr-O | 1.99 | 4.5 | 0.003 |
| | Cr-Cr/Fe | 2.55 | 0.5 | 0.006 |
| | Cr-Cr/Fe | 3.45 | 1.3 | 0.006 |
| | | | | |
| Travis | Pb-O | 2.16 | 0.2 | 0.005 |
| | Pb-O | 2.28 | 1.4 | 0.005 |
| | Pb-O | 2.48 | 0.8 | 0.005 |
| | | | | |
| Mechanicsburg | Pb-O | 2.28 | 1.9 | 0.005 |
| | Pb-O | 2.56 | 1.4 | 0.005 |
| | | | | |
| Portsmouth | Pb-O | 2.26 | 1.4 | 0.01 |
| | Pb-O | 2.47 | 1.3 | 0.01 |
| | | | | |
| Concord | As-O | 1.68 | 4 | 0.0018 |
| | As-MS | * | * | 0.0010 |
| | As-Fe | 2.93 | 0.63 | 0.0046 |
| | As-As | 3.88 | 1 | 0.0018 |
| | | | | |
| Deseret | As-O | 1.68 | 4 | 0.0017 |
| | As-MS | * | * | 0.0010 |
| | | | | |
| Hilo | As-O | 1.69 | 4 | 0.0020 |
| | As-MS | * | * | 0.0010 |
| | As-Al | 3.21 | 1.4 | 0.0070 |
| | | | | |
| Pearl | As-O | 1.75 | 3 | 0.0041 |
| | As-Fe | 2.92 | 0.5 | 0.01 |
| | As-Fe | 3.32 | 0.25 | 0.01 |

* Multiple scattering paths described in text

compounds, but rather associated with organic matter or as a poorly crystalline lead oxide. There is scant EXAFS evidence for association with iron oxides but X-ray fluorescence microprobe maps show a fairly consistent Pb correlation with iron. Cr is present exclusively as Cr(III), and is coordinated by oxygen in two of the Cr-rich samples, with the third sample (Hill AFB) undetermined.

McClellan Air Force Base, Sacramento, CA

Soils at McClellan Air Force Base are fine-grained alfisols with slight acidity and significant organic matter, contaminated with Pb, Cr, and Cd from a former wastewater treatment lagoon.  As shown on Figure A-2, results of X-ray fluorescence microprobe show that Pb is distributed heterogeneously on soil particles. On some soil particles Pb is present with Mn, Zn, and Cr, while on others it coexists with Fe. A low signal to noise ratio and an interference with bismuth at 13419 eV permits limited interpretation of the X-ray absorption spectra. Only the first shell was fit (Table A-1). The local oxygen coordination environment is similar to that in β-PbO (massicot) in that there are contributions from oxygen backscatterers at three distances within the range 2.27 – 2.55 Å. This distal range is slightly expanded relative to massicot (2.22 – 2.48 Å), and the lack of strong second-shell backscattering atoms indicates that Pb is not part of an ordered crystalline structure. The oxygen coordination shell is more complex than what is observed for Pb adsorbed to Fe or Al sesquioxides, where oxygen backscatterers are typically within 2.3 – 2.4 Å of Pb. Based on the high organic content and low pH of the contaminated soil (4.3, compared to 6.1 – 6.6 in the uncontaminated soil), it is possible that Pb is associated with organic matter. The relatively long Pb-O distance (2.46 Å) is consistent with Pb humate at pH 4 (Xia et al., 1997) or Pb acetate (Manceau et al., 1996).

Chromium K-edge EXAFS of the McClellan soil show a local coordination environment similar to that found in chromite, with comparable distances for O, Cr, and Fe backscatterers. However, calculated coordination numbers for Fe and Cr are lower and the XANES  spectrum lacks features characteristic of crystalline chromite, suggesting a disordered long-range coordination environment. A heterogeneous chromium distribution is also suggested by the X-ray fluorescence microprobe maps, which show a non-uniform correlation with Fe. The Cr XANES indicates only Cr(III) is present, indicated by the absence of the significant pre-edge feature associated with Cr(VI) in the Cr K edge XANES spectrum.

## Firing range at Oak Ridge National Laboratory, Tennessee

These soils are highly weathered acidic ultisols, abundant in silt and clay. The bulk X-ray absorption spectrum displays a simple oscillation with no significant second-shell backscattering atoms evident in the Fourier transform (Figure A-3). The local coordination environment is similar to that in the McClellan sample. Based on sample mineralogy, Pb could be adsorbed onto clay minerals or onto sesquioxides, with substantial disorder indicated by the lack of an observed second-shell backscattering contribution.

## Marine Air Corps Station, Cherry Point, NC

This poorly developed entisol, thought to be contaminated from incineration debris, is high in organic matter and very high in iron (about 11 wt.%). The <250 μm size fraction has lower metal concentrations than the <2 mm size fraction, indicating that metals may reside primarily in solid incinerator waste particles. Both Cr and Pb EXAFS were impacted by the presence of other elements (Mn and Bi, respectively), limiting interpretation of these spectra. Two Pb-O distances, 2.34 Å and a smaller contribution at 2.57 Å provided the best fit to the lead L(III) edge EXAFS. Cr is present as Cr(III), as indicated by the absence of the significant pre-edge feature associated with tetrahedrally coordinated Cr(VI) in the Cr K edge XANES spectrum (Peterson et al., 1997). in addition to oxygen, chromium and/or iron contribute to the fit to Cr K edge EXAFS data. Oxygen and the further backscatterer (Cr or Fe) distances are consistent with chromite, but the closer of these backscatterer distances (Cr or Fe) is shorter (2.55 Å, compared to 2.96 Å in chromite), and, like the McClellan sample, the XANES lacks features characteristic of chromite.

## Travis Air Force Base, Fairfield, CA

The silt and clay loam from this site, a former small arms range, is elevated in Pb and other metals, as well as Sb. The X-ray fluorescence microprobe map shows a common distribution pattern for Pb and Zn. On some grains, Pb and Zn are also associated with Fe, Cr, and Mn. In one location, Pb is not associated with any of these other elements. Microbeam XANES analyses on two high-Pb spots are different from one another, despite similarities in elemental associations, suggesting a variety of coordination environments for Pb in this sample.

Three Pb-O distances provided the best fit to the bulk EXAFS data, 2.16, 2.28 and 2.48 Å. The soil environment is interpreted to be highly heterogeneous, leading to a distribution of sorption geometries for Pb. The association with Fe observed on the X-ray fluorescence microprobe map suggests that some of the Pb is likely to be associated with iron oxides, but poorly crystalline lead oxides derived from bullet fragments may predominate.

Naval Support Activity Site, Mechanicsburg, PA

This silty clay ultisol collected from a lead ingot stockpile location is relatively low in metals other than Pb. On the X-ray fluorescence microprobe map, Pb is observed in the same locations as Zn and Se, and not with Fe or Cr. The microbeam XANES spectrum is similar to the bulk spectrum. Although Pb is not in a crystalline compound, its local coordination environment may be similar to that in β-PbO (massicot), similar to the Cherry Point sample. The Fourier transform (not shown) indicates the possibility of a second-neighbor backscattering contributor relatively distant from Pb, at approximately 3.8 Å; however, this spectrum has a low signal:noise ratio and therefore a fit to this second shell could not be constrained. Both the microprobe map and the soil data suggest Pb is not associated with sesquioxides. A mixture of Pb metal or alloy, and poorly crystalline Pb oxides, is a reasonable interpretation in the context of the contamination scenario. Sorption on clay minerals is also a possibility.

Portsmouth Naval Shipyard, Kittery, Maine

Pb in this organic-rich, iron-poor sand/silt inceptisol was introduced from storage of battery cell plates and other materials. In X-ray fluorescence microprobe images, areas of elevated Pb are associated with Cr and/or Bi, and less consistently with Fe. Microbeam XANES support multiple coordination environments for Pb, and no fit was confidently achieved for the bulk EXAFS spectrum beyond the local Pb-O shell, which is similar to the other samples with the best fit by two oxygen distances, 2.26 Å and 2.47 Å.

Hill Air Force Base, Ogden, UT

Chromium contamination at this site, together with cadmium and lead, was left in a sludge drying bed following drinking water treatment. This sandy entisol has high organic matter content (8% TOC). The X-ray absorption near edge structure and first derivative indicate all Cr

is present as Cr(III). The EXAFS spectrum is unusual (Fig. 3) and no fit to this data was performed.

## AS-RICH SOILS

The geochemical controls on As retention in soils are important to health risk assessments and remediation strategies because As toxicity, mobility, and bioavailability are functions of its oxidation state and local chemical environment  (Foster et al., 1998). Arsenic in soil environments is typically found as arsenate, As(V) tetrahedrally coordinated by oxygen at 1.68 Å, or arsenite, As(III) coordinated by three oxygen atoms at 1.75 Å. Organic forms of arsenic can also be present and are usually an indicator of biological activity involving arsenic, or application of organic arsenic compounds such as when poultry litter containing roxarsone (from a feed additive) is spread as a fertilizer. Different forms of arsenic can be distinguished in X-ray absorption spectra by the energy position of the absorption edge, which increases with increasing oxidation state, by the shape of the XANES spectrum, and by the coordination environment assessed from EXAFS data. Results of the synchrotron X-ray studies are presented in Figures A-4 and A-5.

Concord Naval Weapons Site, CA

The silty clay vertisol at the Concord site is impacted by arsenic that was introduced from pesticide application. The X-ray fluorescence microprobe map indicates heterogeneity in As distribution. Most arsenic is associated with Fe, Mn, and Zn, but there are a few grains where As is present without other elements that were included in the mapping scheme. XANES spectra, both in bulk and on a high-As grain, indicate only As(V). The bulk EXAFS is consistent with arsenate adsorbed on iron oxides at a high density, with As-As as well as As-Fe contributions to the fit (Table A-2). Both As-Fe and As-As distances are significantly shorter than in scorodite. The spectra and microprobe maps are not consistent with other minerals that have been found in pesticide-impacted soils, such as schultenite (Cances et al., 2005), arseniosiderite (Cances et al., 2008) or chromated copper arsenate (Bull et al., 2000).
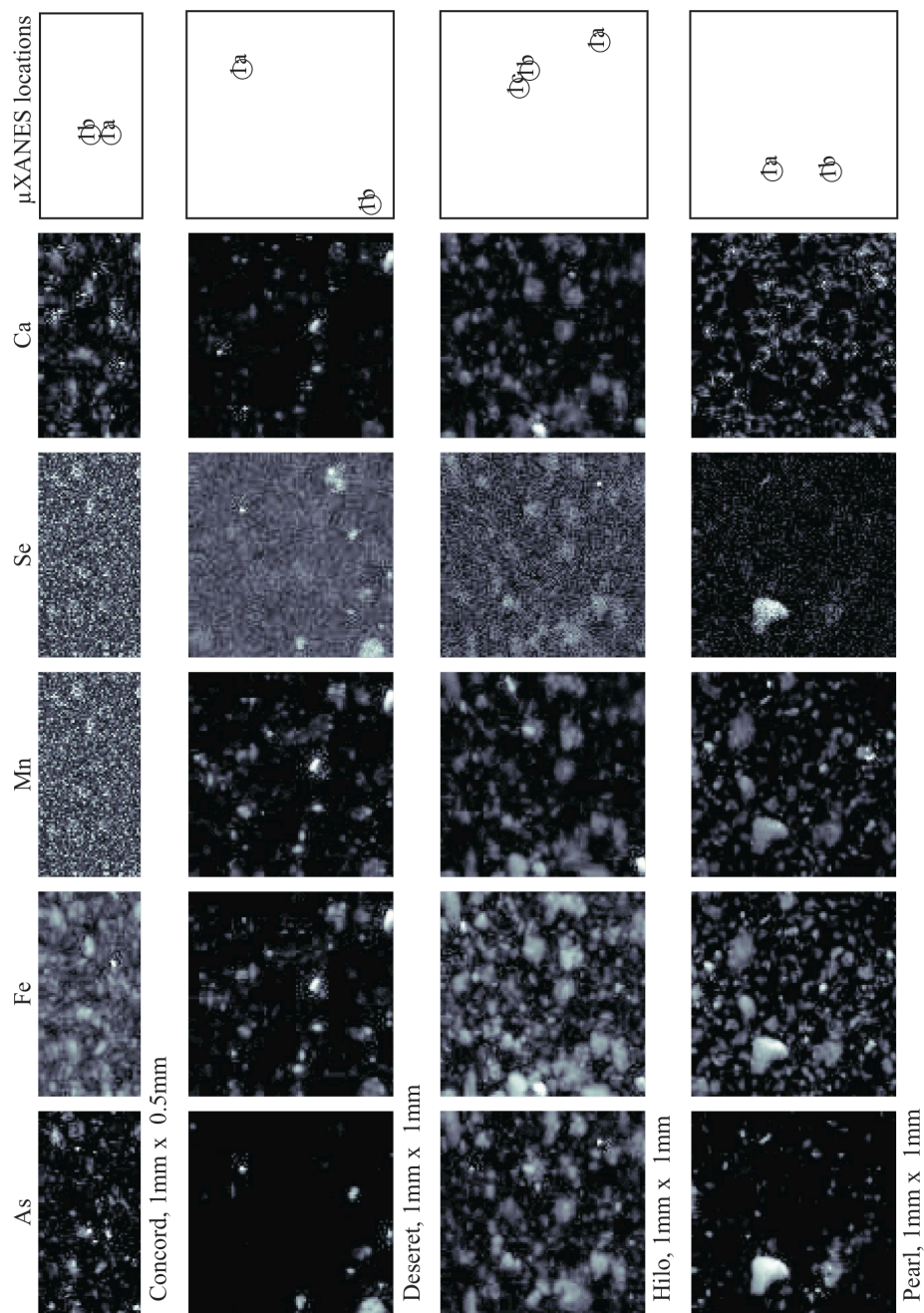
Figure A-4. X-ray fluorescence microprobe maps showing distributions of As, Fe, Mn, Se, and Ca for contaminated soil samples collected from the Concord Naval Weapons site (CA), Deseret Chemical Depot (Tooele, UT), former sugar cane fields near Hilo (HI) and the Pearl Harbor Naval Complex (HI). Locations where microbeam XANES and/or X-ray fluorescence spectra were collected are shown at right.

As K edge XANES and μXANES

XANES First Derivatives

Fits to bulk sample EXAFS
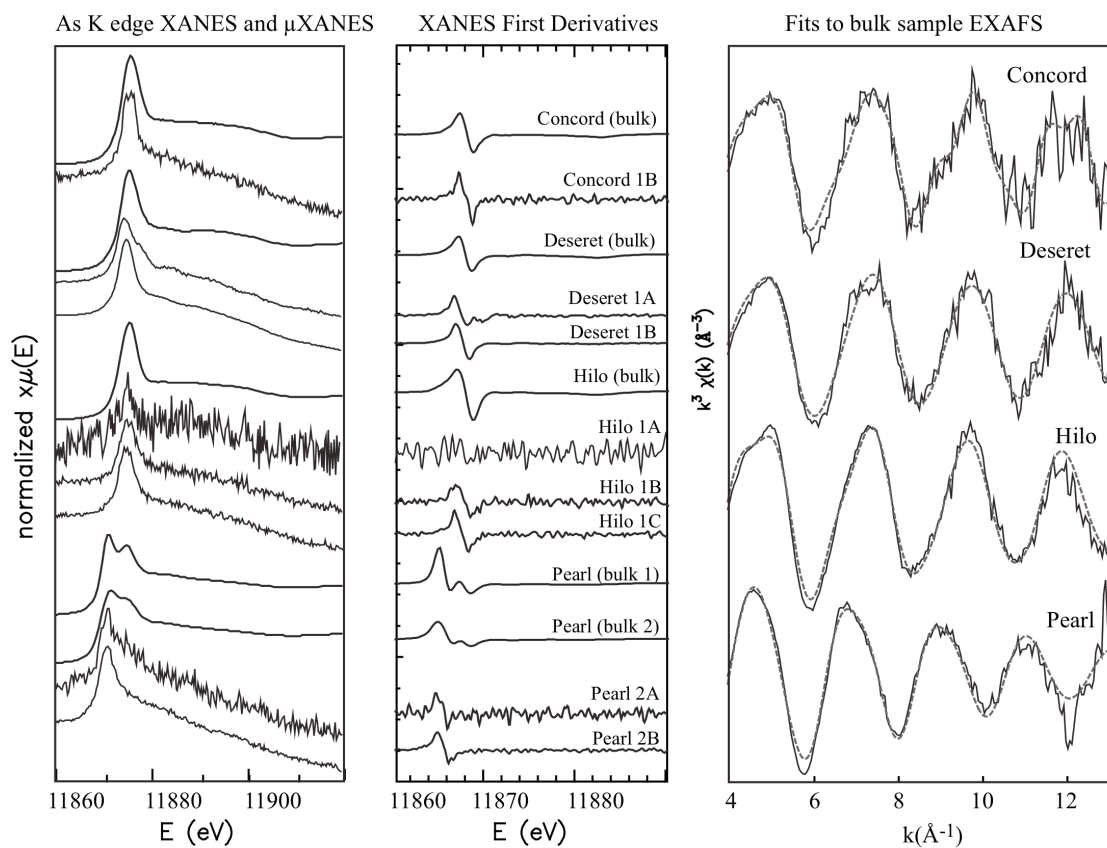
Figure A-5. Arsenic K edge X-ray absorption spectra of contaminated soil samples showing XANES for bulk samples and selected microbeam analyses, first derivatives of XANES spectra, and fits to EXAFS for bulk soil samples. Fit parameters are shown in Table A-2.

<u>Deseret Chemical Depot, Tooele, UT</u>

Arsenic in this silty sand aridisol originates from mine tailings that flooded the site in the 1930s. The pH of this soil is unusually high, 9.3. Arsenic is well correlated with selenium on the microprobe map. Its relationship to iron is variable; some high-arsenic grains are free of iron whereas others are well matched. Including a contribution from multiple scattering in the arsenate tetrahedron considerably improves the fit to the first oscillation in the EXAFS spectrum. Neither iron nor aluminum backscatterers contribute significantly to the pattern.

<u>Former sugar cane fields, Hilo, HI</u>

Similar to the Concord site, arsenic in the Hilo andisol is thought to have originated from pesticide applications in the 1920s - 1940s. The X-ray fluorescence microprobe map shows that arsenic is primarily associated with iron, but there are two locations elevated in arsenic that do not contain iron. One of these is high in selenium. The bulk EXAFS includes a multiple scattering component associated with the arsenate tetrahedron. A good fit is achieved with Al at 3.16 Å as a second-shell backscatterer, consistent with the presence of colloidal allophanes and imogolite in the sample. Ferrihydrite is also ubiquitous and a small second-neighbor contribution from Fe can be fit as well.

<u>Naval Complex, Pearl Harbor, HI</u>

Of the As-bearing soil samples this is the only one in which As is observed as As(III) on the XANES spectra. The bulk sample includes contributions from both As(III) and As(V), but the two microbeam XANES spectra show only As(III). The bulk EXAFS is best fit with 3 oxygen atoms at a distance of 1.75 Å, consistent with arsenite predominance. Iron backscattering at 2.9 Å is consistent with arsenite adsorption on ferrihydrite (Ona-Nguema 2005; Manning et al., 1998). The reducing environment preserving As(III) is consistent with the high organic content of the mollisol.

## SUMMARY

Key observations from the synchrotron X-ray studies are (1) Pb is present as adsorbed divalent ions or as organic complexes, rather than in crystalline compounds, in all of the Pb-rich soil samples; (2) Cr is present as Cr(III), the more stable and less toxic of the two common Cr

oxidation states, in all three Cr-rich soil samples; and (3) Arsenic is present in the more stable and less toxic form, As(V), in three of the four As-rich soil samples, but is present as both As(III) and As(V) in the sample from the Naval Complex at Pearl Harbor. Arsenic appears to occur as an adsorbed complex on iron oxides in the Concord and Pearl samples, and as an adsorbed complex on aluminum oxides in the Hilo soil sample.

In terms of remediation, As strategies will likely require more site-specific approaches versus Pb, which despite soil differences, is more ubiquitously speciated less toxically and with less mobility. No Pb was found to be bound in more immobile and less bioaccessible sulfide phases, meaning that most of the Pb-O in the soils can be liberated under acidic conditions (i.e., in the stomach or in the case of percolating acidic soil/groundwater). The finding that Pb is mobilizable in low pH conditions supported by previous flow-through and leaching experiments performed on the Cherry Point soils (Bang 2004).

## ACKNOWLEDGEMENTS

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# APPENDIX B

# Models for predicting metal toxicity and bioaccumulation in soil invertebrates: Validation of laboratory models using metal-contaminated field soils

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# Models for predicting metal toxicity and bioaccumulation in soil invertebrates: Validation of laboratory models using metal-contaminated field soils

## Introduction

There are thousands of metal-contaminated sites on DoD lands awaiting remediation and closure. The toxic metals lead (Pb), arsenic (As), chromium (Cr), and cadmium (Cd) are of particular concern since they often control risk-based remedial decisions for soils at DoD sites (Exponent, 2001). The ecological risk of metals is directly related to their bioavailability which is the fraction of metal that is absorbed across external membranes (e.g., gut, cuticle, dermis) and enters the body of an organism. The same total metal concentration may result in soil invertebrate responses from complete mortality to 100% survival depending upon the physical/chemical characteristics of the soil (Bradham et al. 2006; Lanno et al. 2004). As such total metal levels in soil may not be the best predictors of toxicity and bioaccumulation. Metal bioavailability can be altered by several soil physical and chemical properties such as Fe-oxide content, organic matter content, and pH. For example, when metal-scavenging manganese (Mn) (Boularbah et al., 1996) or iron (Fe) (Chlopecka and Adriano, 1996) oxyhydroxides are added to soil, metal bioavailability decreased. Certain soil conditions were also found to enhance metal bioavailability. For example, when the soil Fe-oxide content was below 0.5% on a mass basis, the bioavailability of As increased dramatically, particularly in alkaline soils (Yang et al., 2002, 2003). Similarly, for DoD soils low in organic and inorganic carbon, the bioavailability of Cr (III) and Cr (VI) was significantly higher relative to soils that contained higher levels of organic and inorganic carbon (Stewart et al., 2003a, b; Jardine et al., 1999).

Although the concept of metal bioavailability is acknowledged by risk assessors, regulators,

and in guidance documents, there are no consistent, standardized approaches for the application of metal bioavailability considerations in site cleanup actions. EPA's risk assessment guidance implicitly assumes a default relative bioavailability of 100%, or exposure dose is usually measured as total metal concentration, since most bioavailability models are based upon laboratory tests conducted with soils spiked with soluble metal salts. However, this is a conservative assumption, and may overstate the risk posed by less soluble metals in field soils (Davis et al., 1992). Several studies have shown that metals are largely immobilized by soils, and only a small fraction is bioavailable. For example, Banjoko and McGrath (1991) found that most of the zinc (Zn) (78%) present in soil existed in the recalcitrant residual fraction and was not available to maize grown in the soils. Recent research has indicated that reference dose criteria used for soil As and Cr are often highly conservative because the indigenous metal-sequestering properties of many soils can significantly lower the metal bioavailability relative to commonly used default values (Yang et al., 2002, 2003; Stewart et al., 2003a, b). Also, numerous studies have shown that Pb in soil (Freeman et al., 1994; Casteel et al., 1997), mining waste (Dieter et al., 1993; Polak et al., 1996), and aggregate (Cheng et al., 1991; Preslan et al., 1996) is much less bioavailable than more soluble Pb species such as Pb oxide, nitrate, or acetate that are commonly used in toxicological studies.

A range of tools is available to study metal bioavailability, from microscopy, to chemical extractions, to bioassays. Studies have also focused on the application of these techniques specifically to DoD sites (Battelle and Exponent, 2000; Kelley et al., 2002). Based on previous scientific and technical advances in the area of *in vitro* and *in vivo* metal bioavailability in soils, this Environmental Security Technology Certification Program (ESTCP) project was proposed to validate the ability of these techniques to predict metal bioavailability for human and ecological

risk assessment on DoD sites, and to investigate the role of soil properties in controlling metal bioavailability.

This section of the report examines the results of earthworm (*Eisenia andrei*), potworm (*Enchytraeus crypticus, En. albida*), and collembola (*Folsomia candida*) bioassays conducted in soils from metal-contaminated DOD sites with paired reference sites. The goals of this study were: 1) To assess metal bioaccumulation and toxicity to soil invertebrates in a wide range of DOD soils varying in physical and chemical characteristics and metal levels, and 2) To attempt to validate various models relating soil properties or metal extracts to oligochaete metal bioaccumulation and toxicity as a screening tool for predicting metal bioavailability in soils.

**Methods**

*Soil collection*

Eleven DOD sites, differing in geographical location, physical/chemical characteristics, source, and level of metal contamination were selected for soil invertebrate bioassays. For each site, approximately 25 kg of both contaminated soil and site reference soil (the same soil series but uncontaminated, i.e., natural background levels of Cd, Pb, As) were collected. A portable X-Ray Fluorescence meter was used to evaluate approximate metal levels of the soil in the collection area. All soil samples collected from one site were thoroughly mixed to produce one homogenous sample. Prior to soil invertebrate and plant bioassays, soil properties and total metal content were determined.

*Soil preparation and physicochemical characteristics*

Prior to soil invertebrate bioassays, properties of ESTCP soils were determined according

to standard methods of soil analysis (Sparks et al. 1996). Soil pH was measured in both 1:1 soil:deionized water suspension and 1:2 soil: 0.01 M $CaCl_2$ suspension. Electrical conductivity (EC) was measured in 1:1 soil:deionized water suspension. Reactive Al-oxide, Fe-oxide, and Mn-oxide fractions were measured using acid ammonium oxalate extraction.

Webster soil (Ames, IA; 2.4% OC, 35.6% clay, pH 5.5) was used as a lab standard reference soil to monitor test organism performance. Before the test, ESTCP soils (11 contaminated soils paired with 11 reference soils) and Webster soil were sieved to 2.0 mm, air-dried, hydrated with deionized water to achieve a moisture content of 50% of their water-holding capacity (Environment Canada 2004), and left to equilibrate overnight.

*Test organism culture and maintenance*

Sexually mature adult earthworms (*Eisenia andrei*), each with a developed clitellum, weighing approximately 0.3 to 0.5 g wet weight, were obtained from a lab culture maintained in separated dairy solids (SDS; Ohio Agricultural Research and Development Center, Wooster, OH) (Environment Canada 2004). Sexually mature *Enchytraeus albidus* were obtained from a lab culture which was maintained in a mixture of 1:1 Webster soil and potting soil, and was watered and fed with ground oatmeal twice per week. Sexually mature *Enchytraeus crypticus* were obtained from a lab culture which was maintained in Sassafras soil, and was watered and fed with ground oatmeal twice a week (OECD 2003). Adult Collembola (*Folsomia candida*) were obtained from a lab culture which was maintained in a mixture of 9:1 plaster of Paris and powdered charcoal, and was watered and fed with Baker's yeast once per week (ISO 1999).

*Soil invertebrate bioassays*

 *E. andrei* reproduction bioassays were conducted according to standard procedures for earthworm reproduction tests (Environment Canada 2004). Four replicates were prepared for each soil with ten worms placed on the surface of the soil (200 g dry weight) in each test chamber (glass mason jars; 500 ml; Ball, Muncie, IN). Test chambers were sealed with perforated metal lids (one hole, ~2.0 mm, to allow gas exchange) and screw collars. During tests, all the test chambers were maintained under continuous fluorescent lighting at 20±2 ℃. The moisture content of the soil was checked by comparison with initial complete test chamber weights. If weight differed by >5%, deionized water was applied using a spray applicator. The worms were fed with 25 g of SDS once a week. The duration of reproduction test was 56 days. On day 28, adult worms were removed from the soils, counted, placed on moistened filter paper for 24 h to void their gut contents, and then stored in Nalgene bottles (Fisher Scientific, Pittsburgh, PA) and frozen (–70 ℃). Test chambers were returned to the environmental chamber and on day 56, earthworm hatchlings were counted.

 *Enchytraeus* (potworm) bioassays were conducted according to the OECD Guideline 220 (2003). Four replicates were prepared for each soil with ten worms placed on the surface of the soil (20 g dry weight) in each test chamber (glass mason jars; 100 ml; Ball, Muncie, IN). Test chambers were sealed with perforated metal lids (one hole, ~2.0 mm, to allow gas exchange) and screw collars. During the tests, all the test chambers were maintained under continuous fluorescent lighting at 20±2 ℃. The moisture content of the soil was checked by comparison with initial complete test chamber weights. If weight differed by >5%, deionized water was applied using a spray applicator. Potworms were fed with 25 mg of ground oatmeal twice a week. The durations of *En.crypticus* reproduction and *En.albidus* bioaccumulation tests were 28 and 21

days, respectively. For *En.crypticus* reproduction test, on day 14, adult worms were removed from the soils, counted, and then stored in vials and frozen (–70ºC). Test chambers were returned to the environmental chamber and on day 28, hatchlings were counted. For *En.albidus* bioaccumulation tests, adult worms were removed from the soils on day 21, counted, and then stored in vials and frozen (–70ºC).

Collembola bioassays were conducted according to ISO 11267 (1999). Four replicates were prepared for each soil with ten Collembola placed on the surface of the soil (25 g dry weight) in each test chamber (glass mason jars; 100 ml; Ball, Muncie, IN). Test chambers were sealed with a perforated metal lids (one hole, ~2.0 mm, to allow gas exchange) and screw collars. During the tests, all the test chambers were maintained under continuous fluorescent lighting at 20±2ºC. The moisture content of the soil was checked by weight differential and sprayed with deionized water if moisture content decreased more than 5%. Organisms were fed with three granules of Baker's yeast once a week. The duration of Collembola reproduction test was 42 days. On day 28, adult Collembola were removed from the soils, counted, and test chambers returned to the environmental chamber, and on day 42, hatchlings were counted. Adults were pooled and stored in vials at -70ºC until metals analysis.

*Total metal and 0.5 M Ca(NO$_3$)$_2$-extractable metal analysis*

Total metal content of soil was determined using acid digestion (USEPA 3051a, 2007) followed by analysis using high-resolution inductively coupled plasma mass spectrometry (ICP-MS). Soil samples (0.5 g) were oven-dried at 105ºC for 2 h, weighed, mixed with 10 ml of concentrated trace metal-grade HNO$_3$ (Fisher Scientific, Pittsburgh, PA), and digested in a closed Teflon bottle in a microwave oven (Ethos 320; Milestone Inc., Monroe, CT) at 180ºC for

10 min. After cooling at ambient temperature, the solution was diluted to 50 ml with deionized water, and then any residual soil was removed by filtration (0.22 μm). Metal concentrations in the digests were determined with ICP-MS (Elan 6000; Perkin Elmer Sciex., Woodbridge, ON Canada), and reported based upon the dry weight of soil. QA/QC measures included duplicate analyses, metal spikes, blanks, and analyses of standard reference soil ''sandy soil B'' CRM-SA-B, Environmental Express, Mt. Pleasant, SC). Measured metal concentrations of the standard reference soil were within performance acceptance limits.

To determine $Ca(NO_3)_2$-extractable metals, soil samples (1.0 g, dry weight) were placed in 50-ml centrifuge tubes and extracted with 20.0 ml of 0.5 M $Ca(NO_3)_2$ solution. The samples were shaken end-to-end on a reciprocal shaker for 16 h. The soil extracts were centrifuged at 10,000 rpm for 15 min and supernatants decanted and filtered through a 0.45 μm membrane filter. Supernatants were acidified with 1.0 mL of trace metal concentrated HCl and stored in acid-rinsed Nalgene bottles at 4 °C until analysis of metal by ICP-MS.

*Earthworm metal analysis*

For earthworm bioassays, two worms from each replicate were thawed and pooled, oven-dried at 105 ºC to a constant weight, and weighed before the metal analysis. Each earthworm sample was mixed with 10 ml of 25% (v/v) concentrated trace metal-grade $HNO_3$ (Fisher Scientific, Pittsburgh, PA), and digested in a closed Teflon bottle in a microwave oven (Ethos 320; Milestone Inc., Monroe, CT) at 180ºC for 10 min. After cooling at ambient temperature, the solution was made up to 50 ml with deionized water in a volumetric flask. Concentrations of Cd, Cu, Pb, Cr, Ni, Zn, and As in the digests were determined with inductively coupled plasma mass spectroscopy (ICP-MS; Elan 6000; Perkin Elmer Sciex., Woodbridge, ON Canada).

For *Enchytraeus* bioassays, all the worms from two replicates were thawed and pooled

together, oven-dried at 105 ºC to a constant weight, and weighed before the metal analysis. Each

sample was mixed with 10 ml 12.5% (v/v) concentrated trace metal-grade $HNO_3$ (Fisher

Scientific, Pittsburgh, PA), and digested in a closed Teflon bottle in a microwave oven (Ethos

320; Milestone Inc., Monroe, CT) at 180ºC for 10 min. Concentrations of Cd, Cu, Pb, Cr, Ni, Zn,

and As in the digests were determined with ICP-MS (Elan 6000; Perkin Elmer Sciex.,

Woodbridge, ON Canada). All adult collembola from all replicates were pooled and digested for

metals analysis as above, but metal levels were below detection limits.

Quality assurance/quality control (QA/QC) procedures for total metal analysis in organism

tissues included metal analysis of procedural blanks, spikes, and certified reference material

(lobster hepatopancreas, TORT-2, National Research Council, Canada). Measured metal

concentrations of the standard reference tissue were within performance acceptance limits.

*Data analysis*

Mortality, reproduction, and tissue metal concentration data were tabulated in a Microsoft

Excel spreadsheet (Microsoft 2000, 9.0.2812). Student t-tests were used to compare data from

Webster soil and from ESTCP soils to compare parameters between ESTCP soils and the

laboratory reference soil. Student t-tests were also used to compare mean response parameters

betweeen contaminated soils and their corresponding reference soils.

A literature review was performed to assemble empirical models relating earthworm toxicity

endpoints or tissue metal concentrations to total metal soil concentrations and soil properties.

Empirical models developed from previous studies (Lanno and Basta 2003; Yu and Lanno, 2010)

were also applied to predict earthworm metal bioaccumulation. Tissue metal concentrations were

then predicted using these models, and the correlations between predicted values and actual

measured values were tested to validate the application of these models to metal-contaminated

ESTCP field soils. The agreement between the measured and the model-predicted bioavailability

was quantified with the root mean square error (RMSE):

$$RMSE = \left[ \frac{1}{n_d - n_p} \sum_{i=1}^{n_d} \left( B_i - \hat{B}_i \right)^2 \right]^{\frac{1}{2}}$$

where $n_d$ is the numbers of data points, $n_p$ is the number of adjustable parameters (zero when

used in a purely predictive manner as in this project), i is an index, and $B_i$ and $\hat{B}_i$ are the $i$-th

measured and predicted bioavailability, respectively.    The RMSE, the square root of the mean

squared difference between measured and predicted values, is a measure of the average error

between the predicted and measured values.    The goal for models was to produce RMSE $\leq$

25%.


**Results**

        Soil properties and total metal content of the contaminated and reference soils varied

widely (Tables B-1 and B-2). Comparing soil properties over all soils, soil pH ranged from 3.1 to

7.5, Al-oxide content from 345 to 21,344 mg/kg, Fe oxide content from 507 to 25,678 mg/kg,

organic carbon content from 0.2 to 7.8%, cation exchange capacity (CEC) from 2.7 to 39

cmol$_c$/kg, and clay content from 2.5 to 58%. Total metal concentrations ranged from 0.7 to 22

mg/kg for Cd, 12 to 3,069 mg/kg for Pb, 11 to 876 mg/kg for Cr, 3.5 to 561 mg/kg for Ni, 1.7 to

660 mg/kg for As, 30 to 1,889 mg/kg for Zn, and 1.0 to 423 mg/kg for Cu. Such a wide range in

metal concentrations and soil properties provided a reasonable challenge for validating models

predicting metal bioaccumulation by soil invertebrates as related to soil physicochemical

**Table B-1.** Selected properties of ESTCP metal-contaminated soils (C) and reference soils (R).

| Soil | | Soil pH water | Soil pH CaCl$_2$ | EC dS/M | Al$_{ox}$ mg/kg | Fe$_{ox}$ mg/kg | Mn$_{ox}$ mg/kg | Org C % | Total C % | CEC cmol$_c$/kg | Sand % | Silt % | Clay % | WHC % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mechanicsburg | R | 7.5 | 7.1 | 0.3 | 2050 | 2492 | 944 | 1.2 | 1.4 | 9.6 | 9.9 | 50 | 40 | 47 |
| | C | 8.0 | 7.0 | 0.2 | 1615 | 1407 | 290 | 0.6 | 4.5 | 9.7 | 30 | 37 | 34 | 36 |
| Cherry Point | R | 7.4 | 7.0 | 0.4 | 909 | 797 | <25 | 0.8 | 1.9 | 3.9 | 80 | 12 | 7.8 | 32 |
| | C | 5.5 | 5.0 | 0. 9 | 6061 | 7506 | 32 | 3.7 | 4.5 | 9.1 | 80 | 14 | 6.8 | 40 |
| Travis | R | 6.0 | 5.6 | 0.3 | 885 | 4569 | 547 | 1.3 | 1.4 | 11 | 30 | 44 | 26 | 41 |
| | C | 7.0 | 6.5 | 0.3 | 799 | 3088 | 405 | 1.1 | 1.2 | 17 | 48 | 26 | 26 | 36 |
| Concord | R | 6.3 | 5.9 | 0.2 | 1672 | 4519 | 659 | 2.2 | 2.1 | 28 | 20 | 44 | 36 | 48 |
| | C | 6. 7 | 6.2 | 0.1 | 1522 | 3664 | 641 | 3.1 | 3.0 | 28 | 18 | 41 | 41 | 47 |
| McCllelan | R | 6.7 | 6.1 | 0.1 | 487 | 804 | 125 | 0.4 | 0.4 | 12 | 60 | 25 | 15 | 43 |
| | C | 4.3 | 4.3 | 0.3 | 2175 | 4805 | <25 | 4.4 | 4.7 | 13 | 26 | 50 | 24 | 52 |
| Portsmouth | R | 6.2 | 5.7 | 0.2 | 4149 | 2682 | 70 | 1.4 | 1.7 | 2.7 | 87 | 9.6 | 3.9 | 32 |
| | C | 6.2 | 6.0 | 0.1 | 3764 | 5758 | 124 | 1.6 | 2.6 | 2.7 | 89 | 8.5 | 2.5 | 28 |
| Deseret | R | 7.8 | 6.9 | 0.5 | 1207 | 681 | 381 | 0.8 | 1.5 | 13 | 28 | 53 | 19 | 31 |
| | C | 9.3 | 7.5 | 0.5 | 786 | 863 | 313 | 0.7 | 2.3 | 8.4 | 37 | 55 | 8.7 | 35 |
| ORNL | R | 3.8 | 3.1 | 0.2 | 851 | 798 | <25 | 0.2 | 0.2 | 7.9 | 9.0 | 33 | 58 | 44 |
| | C | 4.1 | 3.5 | 0.2 | 388 | 507 | 27 | 0.3 | 0.4 | 2.8 | 46 | 37 | 18 | 41 |
| Pearl | R | 7.7 | 7.5 | 0.9 | 2046 | 1977 | 492 | 0.3 | 2.0 | 39 | 54.7 | 26.9 | 18.4 | 52 |
| | C | 7.3 | 7.3 | 1.0 | 3502 | 44900 | 1014 | 2.3 | 3.3 | 26 | 48.7 | 29.2 | 22.1 | 52 |
| Hilo | R | 4.7 | 4.7 | 1.5 | 5917 | 7535 | 86 | 5.7 | 5.5 | 10 | 72.3 | 17.8 | 2.6 | 40 |
| | C | 5.9 | 5.7 | 0.8 | 21344 | 25678 | 484 | 7.8 | 8.4 | 17 | 61.1 | 25.3 | 7.8 | 45 |
| Webster | R | 6.1 | 5.5 | ---- | 1320 | 2350 | 395 | 2.4 | ---- | 28 | 26 | 32 | 42 | 36 |

properties.

Survival, reproduction, and metal concentrations in the tissues of earthworms (*E. andrei*) from bioassays conducted in contaminated and reference soils are shown in Table B-3. Reduced survival was only observed in Deseret contaminated soil in which the number of live adult worms on day 28 was significantly lower than both Webster soil and the Deseret reference soil. Earthworm survival in all soils tested, except Deseret contaminated soil, was above the validation limit (90% survival) for earthworm bioassays (Environment Canada 2004).

Significantly lower reproduction was observed in all ESTCP soils (contaminated and reference) except for the Concord reference soil in which the number of hatchlings was not significantly different from Webster soil. For about half of the ESTCP soils, reproduction was below the validation limit (30 juveniles per 10 adult worms) for earthworm reproduction bioassays (Environment Canada 2004). *E. andrei* reproduction was significantly lower than in its corresponding reference soil only in Travis soil, suggesting that the decrease in reproduction in most ESTCP soils was due to the effects of the soil matrix, and only in Travis were effects of metal contamination on reproduction observed. Whole-body metal concentrations of *E. andrei* suggest a general trend of increased metal bioaccumulation from contaminated soils. In many cases, metal concentrations in worms exposed to contaminated soils were significantly higher than metal concentrations in worms exposed to either corresponding reference soils or Webster soil, or both. In two cases, Cr for Pearl and As for Cherry Point, metal concentrations in earthworms exposed to contaminated soils were significantly higher than in worms exposed to Webster soil, but significantly lower than in earthworms exposed to corresponding site reference soils. Although these concentrations are statistically significant, the actual differences in concentrations are only about 2.5-fold. For As, whole-body concentrations in *E. andrei* exposed

Table B-2. Total metal content of ESTCP contaminated soils (C) and reference soils (R) along with US EPA Ecological Screening Levels (EcoSSLs) and background soil levels. Cells highlighted in yellow are above US EPA EcoSSLs for the respective metals. Values for metal background levels in soil are take from US EPA EcoSSL documents.

| Soil | | Cd mg/kg | Pb mg/kg | Cr mg/kg | Ni mg/kg | As mg/kg | Zn mg/kg | Cu mg/kg |
|---|---|---|---|---|---|---|---|---|
| Mechanicsburg | R | <1.0 | 33 | 56 | 36 | 17 | 97 | 19 |
| | C | <1.0 | 120 | 39 | 29 | 15 | 98 | 25 |
| Cherry Point | R | <1.0 | 17 | 13 | 3.5 | 1.7 | 32 | <1.0 |
| | C | 19 | 114 | 876 | 78 | 6.9 | 486 | 167 |
| Travis | R | <1.0 | 17 | 43 | 23 | 8.1 | 70 | 19 |
| | C | <1.0 | 2034 | 42 | 29 | 11 | 225 | 148 |
| Concord | R | <1.0 | 16 | 79 | 98 | 7.8 | 101 | 50 |
| | C | <1.0 | 22 | 77 | 92 | 220 | 112 | 54 |
| McCllelan | R | 0.7 | 15 | 126 | 60 | 6.1 | 32 | 14 |
| | C | 22 | 193 | 699 | 87 | 9.9 | 448 | 241 |
| Portsmouth | R | <1.0 | 48 | 14 | 8.4 | 10 | 60 | 12 |
| | C | 1.1 | 3069 | 11 | 62 | 11 | 500 | 185 |
| Deseret | R | <1.0 | 20 | 27 | 17 | 11 | 83 | 15 |
| | C | <1.0 | 19 | 24 | 16 | 438 | 85 | 13 |
| ORNL | R | <1.0 | 12 | 48 | 15 | 14 | 85 | 14 |
| | C | <1.0 | 966 | 16 | 4.2 | 5.0 | 30 | 65 |
| Pearl | R | 1.4 | 13 | 233 | 182 | 4.1 | 133 | 110 |
| | C | 3.6 | 1466 | 185 | 196 | 619 | 1804 | 423 |
| Hilo | R | 1.3 | 153 | 120 | 561 | 22 | 282 | 69 |
| | C | 5.9 | 2134 | 140 | 417 | 660 | 1889 | 224 |
| Webster | R | <1.0 | 0.06 | 0.07 | 0.8 | 4.5 | 3.0 | 41 |
| Invertebrate Eco SSL | | 140 | 1700 | NA | 280 | NA | 120 | 80 |
| Background in U.S. soils | | 0.3-0.5 | 20 | 40-50 | 15-20 | 5-7 | 18-25 | 55 |

in all reference and contaminated ESTCP soils were significantly higher than in *E. andrei* exposed to Webster soil. *E. andrei* survival and reproduction in Webster reference soil were well above validitry criteria, suggesting that observed results were related to test soils and not test organism health or standard test conditions.

Reduced survival was observed in about half of the ESTCP soils in which Enchytraeid survival was also below the validation limit (80% survival) for *En. crypticus* bioassays (OECD Guideline 220, 2003) (Table B-4). However, in only two contaminated soils (Mechanicsburg and Portsmouth), was the number of live adult *En. crypticus* on day 14 significantly lower than in

corresponding site reference soils, suggesting that only in these soils was an effect of metal contamination on survival observed. ORNL soil was intolerable by *En. crypticus* with complete mortality in both reference and contaminated soil. Significantly lower reproduction was observed in about half of the ESTCP soils in which reproduction was also below the validation limit (250 juveniles/10 adult worms) for *En. crypticus* reproduction bioassays (OECD Guideline 220, 2003). However, only in three contaminated soils (Mechanicsburg, Portsmouth, and Pearl) was *En. crypticus* reproduction significantly lower than in corresponding site reference soils, which means only in those soils was an effect of metal contamination on reproduction observed. Whole-body metal concentrations in *En. crypticus* suggest a general trend of increased metal bioaccumulation from the most contaminated soils where whole-body metal concentrations in potworms exposed to contaminated soils were significantly higher than potworms exposed to corresponding site reference soils or Webster soil, or both. In soils where metal concentrations were only marginally higher than in site reference soils, no increase in metal concentrations was observed. *En.crypticus* survival and reproduction in Webster reference soil were well above validitry criteria, suggesting that observed results were related to test soils and not test organism health or standard test conditions.

Collembola (*F. candida*) survival was reduced in seven ESTCP soils in six of which survival was also below the validation limit (80% survival) for Collembola bioassays (Table B-5) (ISO 11267 1999). However, in only three contaminated soils (Travis, Concord, ORNL) was the number of live adult Collembola on day 28 significantly lower than in corresponding reference soils, suggesting possible effects of metal contamination. Significantly lower reproduction was observed in about half of the ESTCP soils, but only in Cherry Point contaminated soil was reproduction below the validation limit (100 juveniles reproduced by 10 adult Collembola) for

**Table B-3.** *E. andrei* survival, reproduction, and tissue metal concentrations of Webster soil, ESTCP contaminated soils (C) and reference soils (R).

| Soil | | Survival on Day 28 | Hatchlings on Day 56 | Tissue concentration on Day 28 (mg/kg) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cd | Cu | Pb | Zn | Cr | Ni | As |
| Webster | | 10 | 77 | 3.9 | 35 | 1.1 | 179 | 2.5 | 3.4 | 0.7 |
| Mechanicsburg | R | 9.8 | 28[24] | 2.0 | 35 | 1.8 | 174 | 3.6 | 2.7 | 3.6* |
| | C | 9.8 | 31[2] | 2.0 | 33 | 6.6** | 156 | 3.7 | 2.7 | 8.0** |
| Cherry Point | R | 9.5 | 11[24] | 1.3 | 22 | 0.9 | 102 | 1.9 | 0.9 | 5.0* |
| | C | 10 | 36[2] | 40** | 64** | 9.5** | 187** | 65** | 15** | 1.7[1] |
| Travis | R | 10 | 27[24] | 2.0 | 31 | 1.8 | 155 | 3.2 | 4.2 | 8.8* |
| | C | 10 | 20[234] | 1.8 | 508** | 143** | 170** | 3.5 | 3.2 | 17** |
| Concord | R | 9.5 | 76 | 2.4 | 37 | 1.6 | 147 | 5.2* | 11* | 1.7 |
| | C | 9.3 | 65[2] | 2.0 | 27 | 0.4 | 135 | 2.0 | 4.1 | 110** |
| McCllelan | R | 9.8 | 57[2] | 3.3 | 34 | 0.7 | 153 | 2.1 | 2.8 | 2.0 |
| | C | 9.5 | 42[2] | 86** | 63** | 5.3** | 197** | 18** | 21** | 4.1** |
| Portsmouth | R | 9.8 | 53[2] | 3.5 | 38 | 9.6* | 154 | 3.6 | 2.9 | 4.0* |
| | C | 9.5 | 44[2] | 3.3 | 62** | 295** | 214** | 3.1 | 4.8 | 4.5 |
| Deseret | R | 9.5 | 17[24] | 3.5 | 19 | 1.5 | 137 | 2.6 | 2.2 | 5.5* |
| | C | 7[234] | 11[24] | 8.7** | 38** | 4.1** | 257** | 4.8** | 6.2** | 138** |
| ORNL | R | 9.8 | 10[24] | 2.2 | 26 | 1.5 | 176 | 1.5 | 1.9 | 1.9 |
| | C | 9.3 | 7.0[24] | 1.5 | 36** | 490** | 210** | 1.6 | 1.5 | 2.5* |
| Pearl | R | 9.8 | 11[24] | 2.5 | 30 | 1.8 | 169 | 17* | 16* | 1.6 |
| | C | 10 | 31[2] | 5.0 | 44 | 85** | 251** | 8.8[1] | 21* | 126** |
| Hilo | R | 10 | 3.5[24] | 2.0 | 21 | 22* | 153 | 15* | 40* | 5.8* |
| | C | 10 | 19[24] | 2.9 | 51** | 384** | 372** | 20 | 37 | 143** |

For tissue metal concentration:

* Significantly higher ($\alpha$ =0.05) than Webster soil

** Significantly higher ($\alpha$ =0.05) than corresponding reference soil and Webster soil - blue

[1] Significantly lower ($\alpha$ =0.05) than corresponding reference soil, but significantly higher ($\alpha$ =0.05) than Webster soil

For survival and reproduction:

[2] Significantly lower ($\alpha$ =0.05) than Webster soil – highlighted in purple

[3] Significantly lower ($\alpha$ =0.05) than corresponding reference soil - highlighted in green

[4] Below the validation limit for bioassay set by Environment Canada (2004)

B-15

**Table B-4.** *En. crypticus* survival, reproduction, and whole-body metal concentrations in worms exposed to Webster reference soil, ESTCP contaminated soils (C) and reference soils (R).

| Soil | | Survival on Day 14 | Hatchlings on Day 28 | Whole-body concentration on Day 14 (mg/kg) | | | | | | |
|------|---|------|------|------|------|------|------|------|------|------|
| | | | | Cd | Cu | Pb | Zn | Cr | Ni | As |
| Webster | | 9.7 | 406 | 3.1 | 20 | 5.0 | 369 | 8.6 | 11 | 1.9 |
| Mechanicsburg | R | 6.5[24] | 172[24] | 5.4 | 14 | 4.3 | 447 | 3.1 | 4.7 | 0.9 |
| | C | 1.8[234] | 80[234] | 9.0** | 19 | 6.6 | 498 | 7.9*** | 12*** | 7.2** |
| Cherry Point | R | 4.8[24] | 136[24] | 2.5 | 21 | 5.3 | 301 | 7.1 | 6.1 | 7.8* |
| | C | 8.3 | 322 | 34** | 45** | 52** | 338 | 295** | 30** | 2.7 |
| Travis | R | 8.5 | 224[24] | 8.1* | 21 | 11* | 334 | 17* | 8.5 | 1.4 |
| | C | 5[24] | 226[24] | 2.7 | 24 | 124** | 382 | 3.9 | 11 | 1.7 |
| Concord | R | 10 | 298 | 4.3 | 19 | 4.4 | 266 | 7.8 | 13 | 2.4 |
| | C | 7.5[24] | 203[24] | 2.9 | 14 | 5.4 | 351 | 6.5 | 14 | 17** |
| McCllelan | R | 10 | 337 | 1.3 | 12 | 1.6 | 166 | 3.3 | 3.7 | 1.7 |
| | C | 8.5 | 306 | 46** | 53** | 24** | 518** | 66** | 60** | 13** |
| Portsmouth | R | 7.8[24] | 245[24] | 2.8 | 13 | 20 | 285 | 3.7 | 2.3 | 1.8 |
| | C | 1.8[234] | 0[234] | 2.7 | 14 | 169** | 827** | 5.8 | 3.9 | 1.9 |
| Deseret | R | 10 | 331 | 2.2 | 9.4 | 12* | 183 | 1.6 | 2.5 | 1.8 |
| | C | 10 | 299 | 4.4*** | 6.1 | 4.7 | 193 | 10*** | 12*** | 63** |
| ORNL | R | 0[24] | 0[24] | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| | C | 0[24] | 0[24] | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| Pearl | R | 10 | 393 | 0.3 | 11 | 6.1 | 289 | 18* | 13 | 1.3 |
| | C | 10 | 231[234] | 4.0*** | 142** | 421** | 801** | 58** | 55** | 213** |
| Hilo | R | 5.5[24] | 249[24] | 2.1 | 13 | 78* | 158 | 72* | 118* | 25* |
| | C | 10 | 520 | 6.0** | 38** | 1458** | 687** | 68* | 118* | 510** |

For tissue metal concentration:

\* Significantly higher ( α =0.05) than Webster soil

\*\* Significantly higher ( α =0.05) than corresponding reference soil and Webster soil – highlighted in blue

\*\*\* Significantly higher ( α =0.05) than corresponding reference soil

For survival and reproduction:

[2]   Significantly lower ( α =0.05) than Webster soil – highlighted in purple

[3]   Significantly lower ( α =0.05) than corresponding reference soil – highlighted in green

[4]   Below the validation limit for bioassay set by the OECD Guideline 220 (2003)

**Table B-6.** *Folsomia candida* survival and reproduction of Webster soil, ESTCP contaminated soils (C) and reference soils (R).

| Soil | | Survival on Day 28 | Hatchlings on Day 42 |
|---|---|---|---|
| Webster | | 9.9 | 529 |
| Mechanicsburg | R | 10 | 435 |
| | C | 9.5 | 251[23] |
| Cherry Point | R | 10 | 331[2] |
| | C | 10 | 97[234] |
| Travis | R | 10 | 514 |
| | C | 6.3[234] | 365[23] |
| Concord | R | 10 | 482 |
| | C | 5.5[234] | 372[23] |
| McCllelan | R | 9.5 | 513 |
| | C | 9.8 | 367[23] |
| Portsmouth | R | 9.3 | 308[2] |
| | C | 8.8 | 365[2] |
| Deseret | R | 5.5[24] | 408 |
| | C | 10 | 425 |
| ORNL | R | 10 | 243[2] |
| | C | 7.0[234] | 279[2] |
| Pearl | R | 10 | 452 |
| | C | 9.0 | 582 |
| Hilo | R | 7.0[24] | 400 |
| | C | 8.5[2] | 249[2] |

For survival and reproduction:

[2]  Significantly lower ( α =0.05) than Webster soil.

[3]   Significantly lower ( α =0.05) than corresponding reference soil – highlighted in green

[4]  Below the validation limit for bioassay set by ISO 11267 (1999).

Collembola reproduction bioassays (ISO 11267, 1999). In five contaminated soils (Mechanicsburg, Cherry Point, Travis, Concord, McCllelan), Collembola reproduction was significantly lower than in their corresponding reference soils, suggesting effects of metal contamination in these soils. *F. candida* survival and reproduction in Webster reference soil were well above validitry criteria, suggesting that observed results were related to test soils and not test organism health or standard test conditions.

In order to quantitatively assess the factors that affect metal bioaccumulation and toxicity to *E. andrei*, a literature review was conducted to assemble empirical models relating earthworm toxicity endpoints or whole-organism metal concentrations to total metal concentrations in soils and soil properties. One hundred and thirty-four models from 18 studies were collected with two models for As, 31 for Cd, one for Cr, 30 for Cu, three for Ni, 36 for Pb, and 31 for Zn. No models relating toxicity to metal concentrations and soil physicochemical characteristics were found, and all the models were bioaccumulation models using either bioaccumulation factor (BAF) or whole-organism metal concentration as a dependent variable. Each model had its own specific earthworm species and ranges of soil parameters that it described. All the models are listed in Appendix B-1 with a summary of the studies from which these models were collected.

Data on soil properties and total metal content of ESTCP soils were applied to these models, and predicted values of BAF or whole-organism metal concentrations were obtained. Then, the correlation between predicted values and values actually measured by bioassay was examined, and the correlation coefficients ($R^2$) were listed in Appendix B-1.

There were only two models for As, but one of them provided a good fit to the ESTCP data ($R^2 = 0.91$, RMSE = 24.2%, Figure B-1) with total soil As concentration accounting for about 91% of the variability observed in earthworm As concentrations. This correlation should be interpreted with caution since there are two groups of points, one including very low levels of As in soil while the other group of data points represent As contaminated sites. The grouping of data points was similar for Cr (Figure B-2) and Ni (FigureB- 3), both of which had just a few models in the literature, especially models relating bioaccumulation to soil property parameters. However, the total soil concentrations of these metals accounted for a large portion of variability in earthworm tissue concentrations of these metals, 73% for Cr and 88% for Ni. Although the

regression model fit was good, predicted Ni concentrations in earthworms were much higher

than measured values (RMSE = 689%), resulting in poor predictive ability for this model. This

may be a result of extrapolation since the range of Ni concentrations in soils from which the

model was developed was very narrow (1.3-2.4 mg/kg) while ESTCP soils had a much wider

range of Ni concentrations (3.5 to 561 mg/kg). However, for Cr, the model under-predicted Cr

bioaccumulation but, with a RMSE = 13.6%, this model provided good predictive agreement.
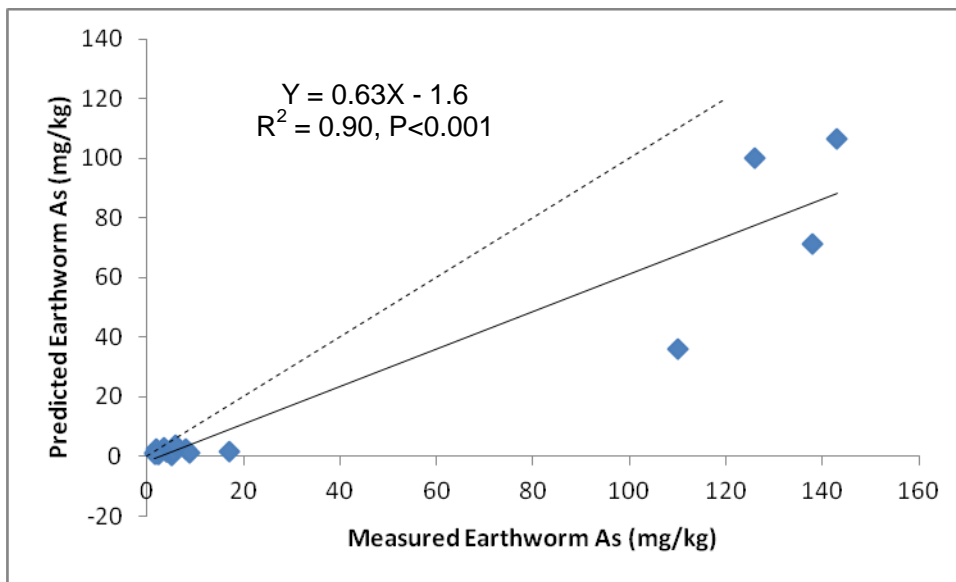


Figure B-1. Relationship between predicted and measured As concentrations in earthworms

(*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: $\ln As_{ew}=0.9884*\ln$

$As_s-1.747$ (Sample et al. 1998). Dotted line is 1:1 predicted:measured As.
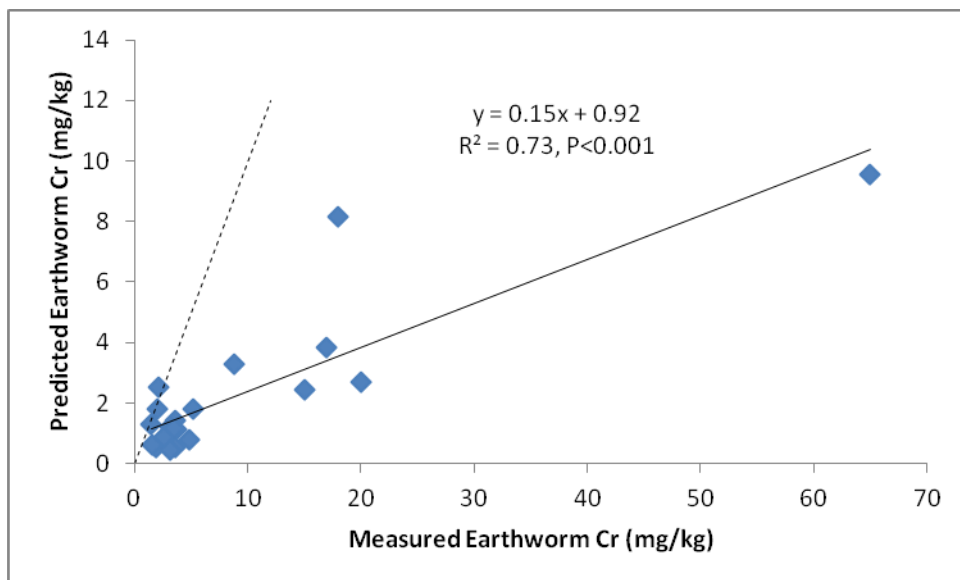
Figure B- 2. Relationship between predicted and measured Cr concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: $\log Cr_{ew}=0.69*\log Cr_s-1.05$ (Peijnenburg et al. 1999a). Dotted line is 1:1 predicted:measured Cr.



Figure B-3. Relationship between predicted and measured Ni concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: $\log Ni_{ew}=0.98*\log Ni_s+0.67$ (Neuhauser et al. 1995). Dotted line is 1:1 predicted:measured Ni.

There were 31 models for Cd, and 16 of them used total soil Cd concentration as the only parameter in the model. Among these models, the largest $R^2$ value was 0.82, suggesting that total soil Cd concentration could account for about 82% of the variability in earthworm Cd concentrations. The best fitting model was a model using total soil Cd concentration, soil pH, and organic matter content (OM) as parameters, and these three parameters could account for about 98% of the variability in earthworm Cd concentrations (Figure B-4). Although such a high $R^2$ value was partly due to the nature of our data (only eight data points were included because total soil Cd concentrations in some ESTCP soils were below detection limits, and of the eight data points, most were in the low concentration range), that model was still the best compared to the other 30 models. Although this model fit the ESTCP data very well, it had a poor predictive capacity with a RMSE of 106% .



Figure B-4. Relationship between predicted and measured Cd concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: ln $Cd_{ew}$=6.018+0.787*ln $Cd_s$-0.106*OM-0.402*pH (Ma et al. 1983). Dotted line is 1:1 predicted:measured Cd.

One issue related to these empirical, multiple-regression type models is that they do not consider metal uptake kinetics in predicting bioaccumulation. Cd uptake kinetics have been shown to be linear over short time periods (as in these ESTCP tests) and not reach steady state during experimental exposure (Spurgeon and Hopkin 1999; Sheppard et al. 1997; Neuhauser et al. 1995; van Gestel et al. 1993), but recent models have been developed that describe Cd uptake kinetics and estimate steady-state concentrations over longer time periods (Yu and Lanno, 2010). In this recent study, one of the exposure concentrations was 20 mg Cd/kg in Webster soil, resulting in the following model:

$$C_w = 9.32*e^{-0.008*28} + Cd_s*0.052/0.008*(1- e^{-0.008*28})$$

where $C_w$ is the total Cd concentration in the earthworm and $C_s$ is the total Cd concentration in the soil. Application of this model to predicting steady-state Cd bioaccumulation by *E.andrei* in two ESTCP soils (Cherry Point and McLellan) that had total Cd concentrations similar to that used in model development (19 and 22 mg Cd/kg, respectively) resulted in a reasonable approximation for Cd uptake by *E. andrei* exposed to Cherry Point soil (40 mg Cd/kg measured vs 32 mg Cd/kg predicted), but under-predicted Cd bioaccumulation in McLellan soil (86 mg Cd/kg measured vs 36 mg Cd/kg predicted). When the remainder of the ESTCP soils were included in the model estimation, the kinetics model provided accepatable predictive capacity with a RMSE of 19%, based upon eight data points.

There were 36 models for Pb, and 20 of them used total soil Pb concentration as the only parameter in the model. Among these models, the largest $R^2$ value was 0.62 suggesting that total soil Pb concentration could account for about 62% of the variability in earthworm Pb concentrations at most. Including soil pH as another parameter in the model significantly
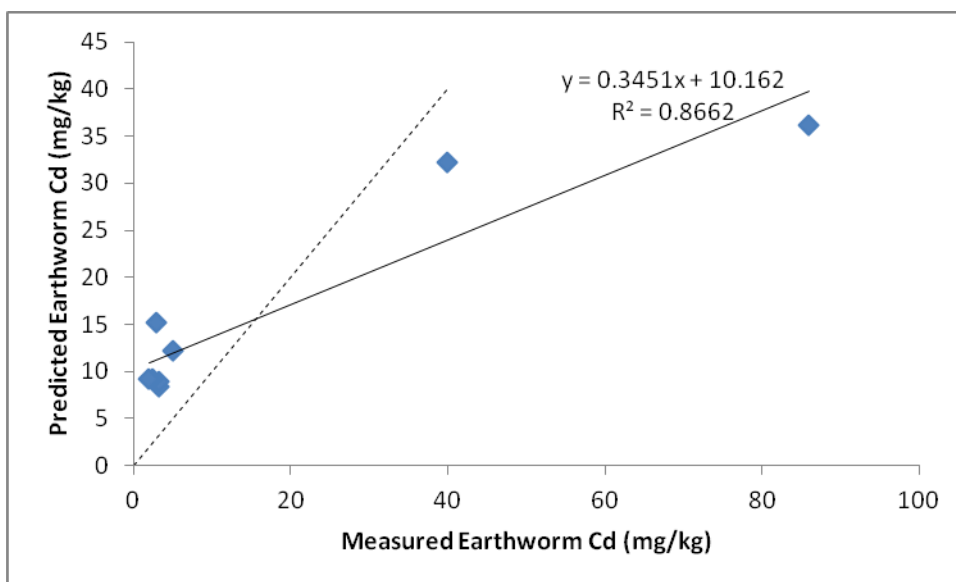
Figure B-5. Relationship between predicted and measured Cd concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: $C_w = 9.32 \ast e^{-0.008 \ast 28} + Cd_s \ast 0.052/0.008 \ast (1 - e^{-0.008 \ast 28})$ (Yu and Lanno 2010). Dotted line is 1:1 predicted:measured Pb.

improved the explanatory ability of the model, with $R^2$ values increasing to around 0.9, but adding OM or CEC to the model did not improve the relationship significantly. The best fitting model included total soil Pb concentration and soil pH as parameters and accounted for about 95% of the variability in earthworm Pb concentrations (Figure B-6). However, the model consistently over-predicted Pb bioaccumulation, with RMSE of 272%.

There were 30 models for Cu, and 23 of them incorporated total soil Cu concentration as the only parameter in the model. Among these models, the best accounted for about 45% of the variability in earthworm Cu concentrations (Figure B-7), under-predicted Cu bioaccumulation (RMSE = 24.7%). Adding soil property parameters such as soil pH or OM to the model did not improve the model significantly. The situation was the same for Zn. There were 31 models for Zn, and 22 of them used total soil Zn concentration as the only parameter in the model. Total soil Zn

concentration could account for about 62% of the variability in earthworm Zn concentrations, but
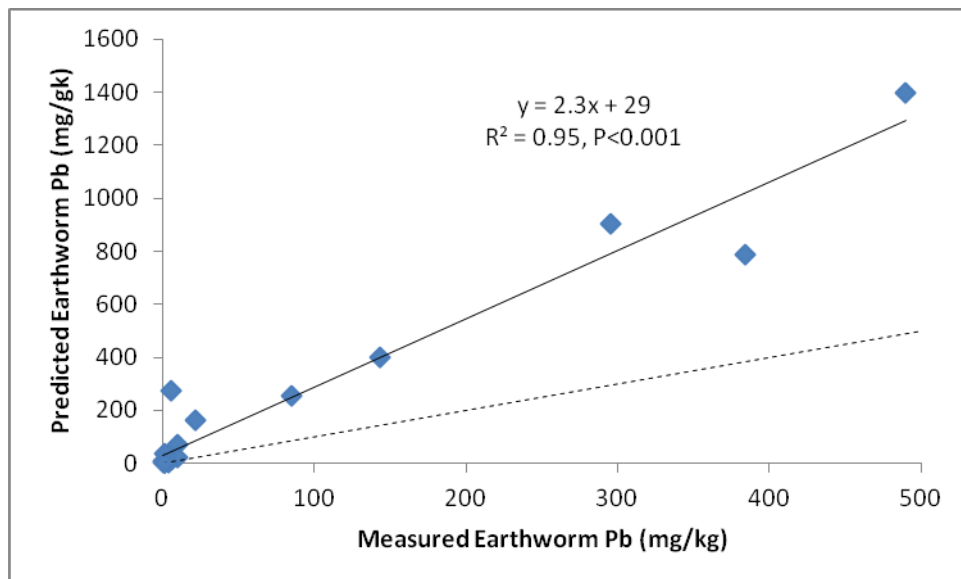


Figure B-6. Relationship between predicted and measured Pb concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: log $Pb_{ew}$=2.65+0.897*log $Pb_s$-3.56*log pH (Corp and Morgan 1991). Dotted line is 1:1 predicted:measured Pb.
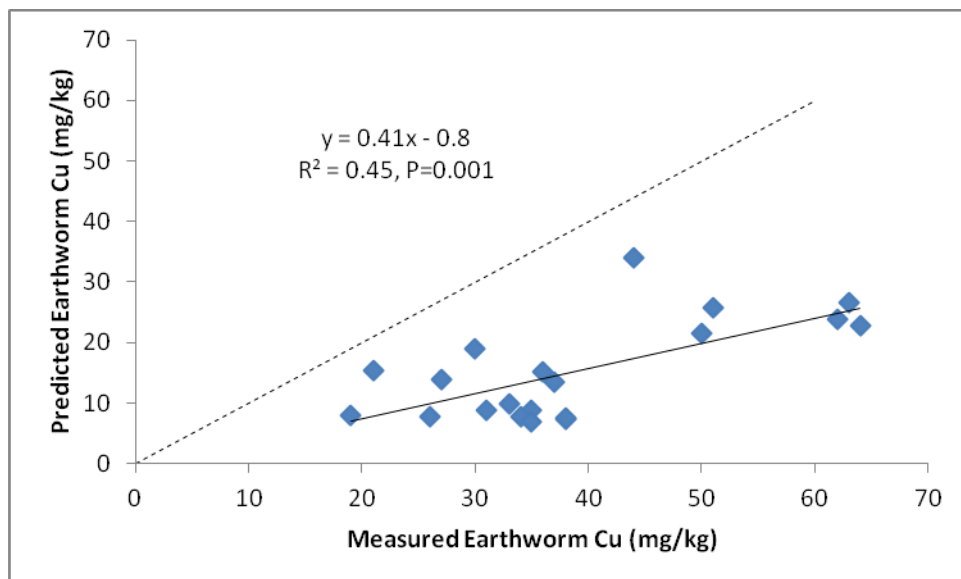


Figure B-7. Relationship between predicted and measured Cu concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: log $Cu_{ew}$=0.435*log $Cu_s$+0.39 (Morgan and Morgan 1988). Dotted line is 1:1 predicted:measured Cu.
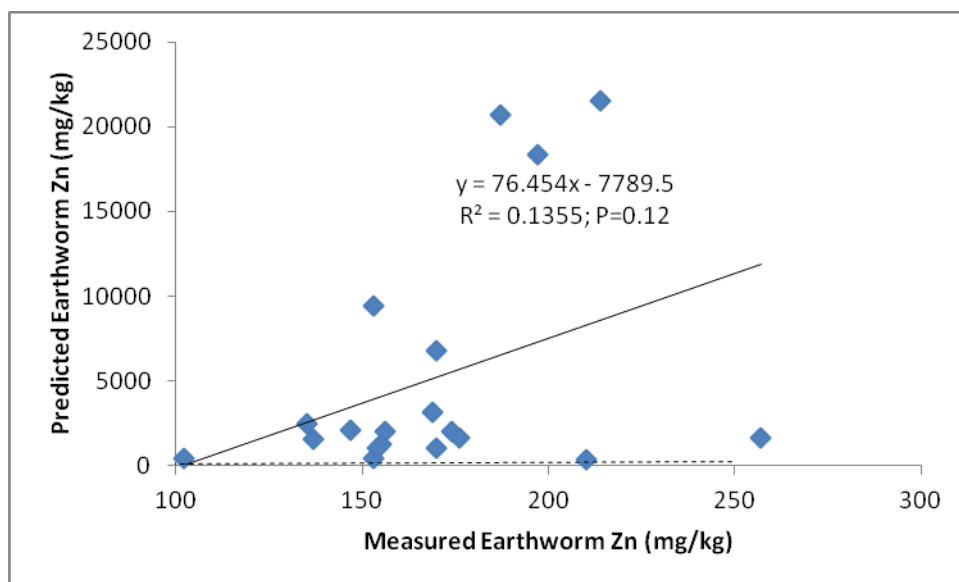
Figure B-8. Relationship between predicted and measured Zn concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: log $Zn_{ew}$=1.45*log $Zn_s$+0.42 (Peijnenburg et al. 1999a). Dotted line is 1:1 predicted:measured Zn.

this model over-predicted extremely high Zn concentrations in earthworms (RMSE = 590%) (Figure B-8). Adding soil property parameters such as soil pH or OM to the model did not significantly improve the model.

In order to quantitatively assess the factors that affect metal accumulation and toxicity to *En. crypticus*, a literature review was performed to assemble empirical models relating Enchytraeid toxicity endpoints or tissue metal concentrations to total metal concentrations in soils and soil properties. No toxicity models were found, and three bioaccumulation models using
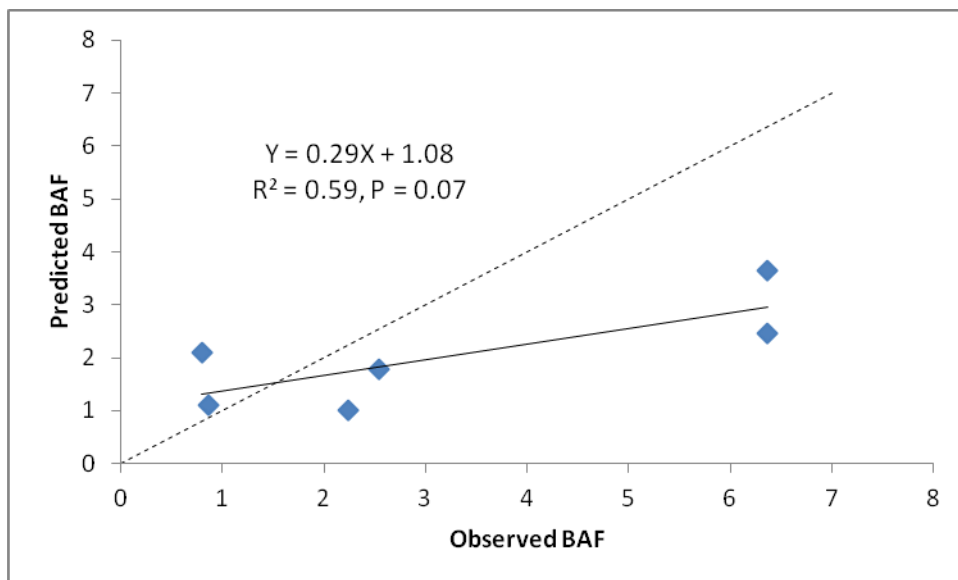
Figure B-9. Relationship between Cd BAF predicted by model: log BAF=1.17-0.92*log Clay (Peijnenburg et al. 1999b) and Cd BAF measured in *En. crypticus* exposed to ESTCP soils. Dotted line represents 1:1 predicted:observed.

bioaccumulation factor (BAF) as a dependent variable from Peijnenburg et al. (1999) were assembled with for Cd, Pb, and Zn. Data on soil properties and total metal content of ESTCP soils were applied to these models, and predicted values of BAF were obtained. The relationship between predicted BAFs and measured BAFs for enchytraeids exposed to ESTCP soils (Figures B-9-B-11) was very weak for Pb and Zn, but provided a good fit for Cd (RMSE = 21%), based upon four data points.
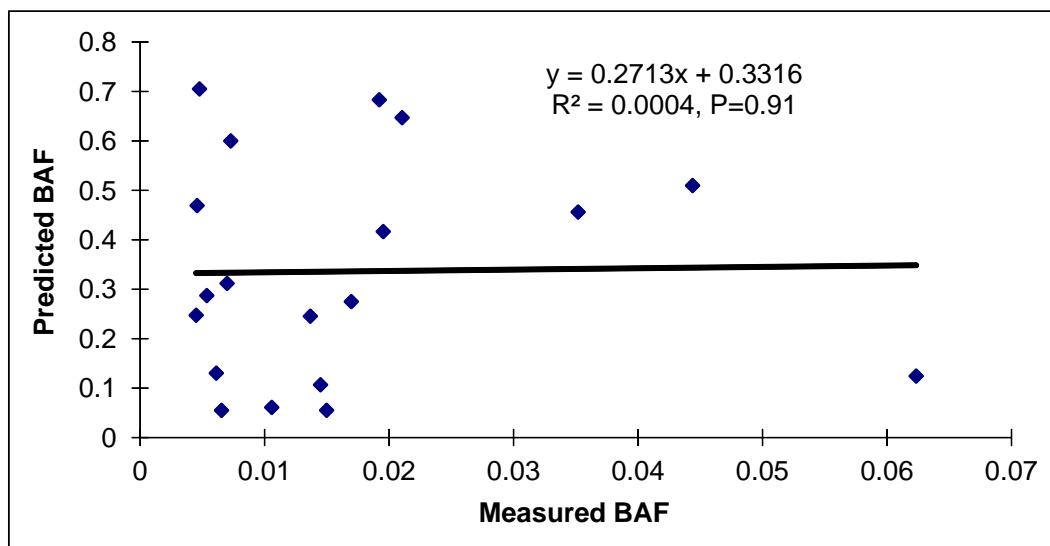
Figure B-10. Relationship between Pb BAF predicted by model: log BAF=0.35-0.36*pH

(Peijnenburg et al. 1999b) and Pb BAF measured in *En. crypticus* exposed to ESTCP soils.
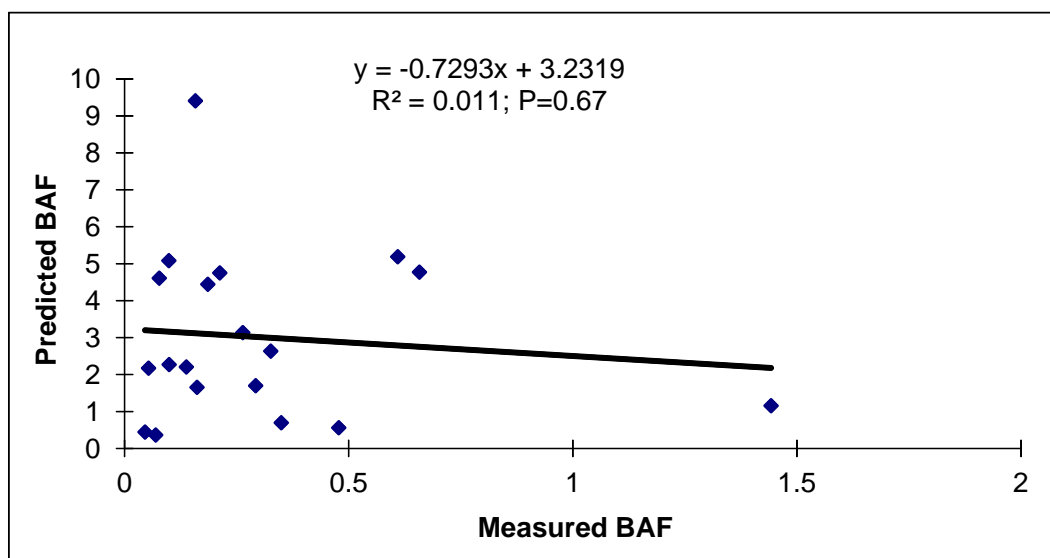


Figure B-11. Relationship between Zn BAF predicted by model: log

BAF=3.47-0.46*pH-0.67*log $Al_{ox}$ (Peijnenburg et al. 1999b) and Zn BAF measured in *En.*

*crypticus* exposed to ESTCP soils.

A literature search was conducted for models relating metal toxicity or bioaccumulation in

Collembola to total metal concentrations and soil physical/chemical properties, but none was

found.

For many of the ESTCP contaminated soils, 0.5 M $Ca(NO_3)_2$-extractable metal levels were

below limits of quantitation (Table B-6). No As or Cr was detected in 0.5 M $Ca(NO_3)_2$ extracts

from any of the soils and Cd was only detected in Cherry Point and McCllelan soils. Pb was

detected in 0.5 M $Ca(NO_3)_2$ extracts from five soils (Hilo, McCllelan, ORNL, Portsmouth,

Travis) and extractable Pb ranged from 2-62% of measured total metal levels. Zn was detected in

0.5 M $Ca(NO_3)_2$ extracts from four soils (Cherry Point, Hilo, McCllelan, Portsmouth) and

extractable Zn ranged from 10-38% of measured total metal levels.

**Table B-6.** Extractable (0.5 M $Ca(NO_3)_2$) metal content (mg/kg) of ESTCP contaminated soils.
Values in parentheses are the percent of total metal that is extractable with a 0.5 M $Ca(NO_3)_2$
solution.

|  | As | Cd | Cr | Pb | Zn |
|---|---|---|---|---|---|
| Concord | <20 | <2 | <2 | <2 | <20 |
| Cherry Point | <20 | 16.6(88%) | <2 | <2 | 109 (22%) |
| Deseret | <20 | <2 | <2 | <2 | <20 |
| Hilo | <20 | <2 | <2 | 33 (1.5%) | 535 (28%) |
| McCllelan | <20 | 15.5(71%) | <2 | 27(14%) | 171 (38%) |
| Mechanicsburg | <20 | <2 | <2 | <2 | <20 |
| ORNL | <20 | <2 | <2 | 603(62%) | <20 |
| Pearl | <20 | <2 | <2 | <2 | <20 |
| Portsmouth | <20 | <2 | <2 | 507(17%) | 52 (10%) |
| Travis | <20 | <2 | <2 | 48 (2.4%) | <20 |

Models developed in previous studies (Lanno and Basta 2003) that established correlations

between 0.5 M $Ca(NO_3)_2$-extractable metals and bioaccumulation by earthworms were also used

to predict metal bioaccumulation by earthworms exposed to ESTCP soils. Lanno and Basta

(2003) models were based upon 22 soils differing in physicochemical properties that were

amended with one concentration of either Pb or Zn, or three concentrations of Cd, allowing

equilibration with the soils to determine the bioavailable fraction of metals. The model in Figure

B-12 relating log Pb concentration in earthworms to log 0.5 M $Ca(NO_3)_2$-extractable Pb ($R^2$ –

0.39) was based upon soils amended with 2,000 mg/kg Pb. Except for McCllelan, this model

tended to under-predict Pb bioaccumulation by *E. andrei* (Figure B-13; $R^2$ – 0.43; RMSE –

161%).
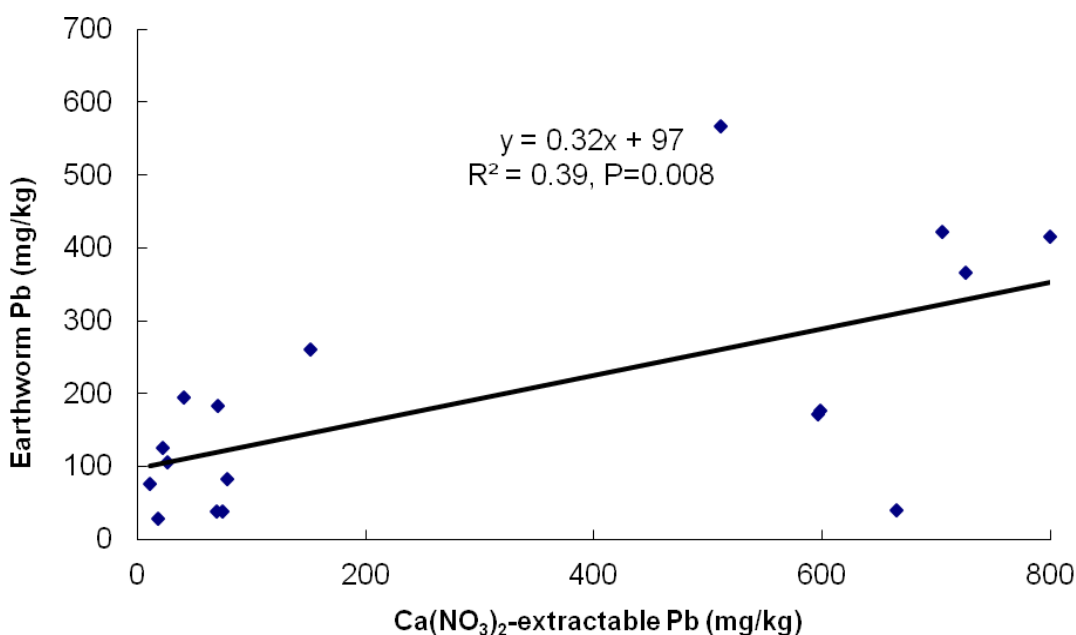


Figure B-12. Relationship between Pb concentrations in earthworms and 0.5 M

$Ca(NO_3)_2$-extractable Pb. Model was developed using 22 soils differing in physicochemical
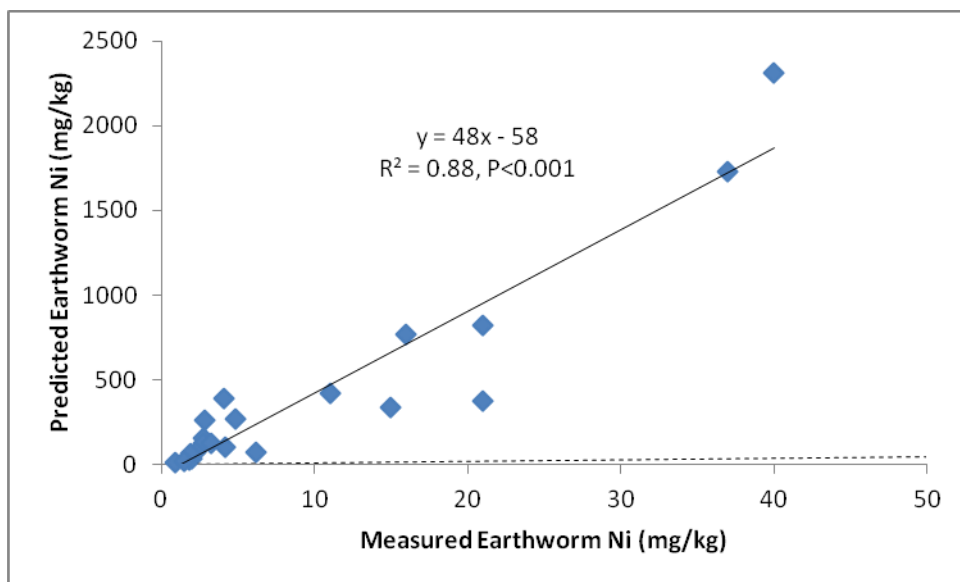
characteristics amended with 2,000 mg/kg Pb.
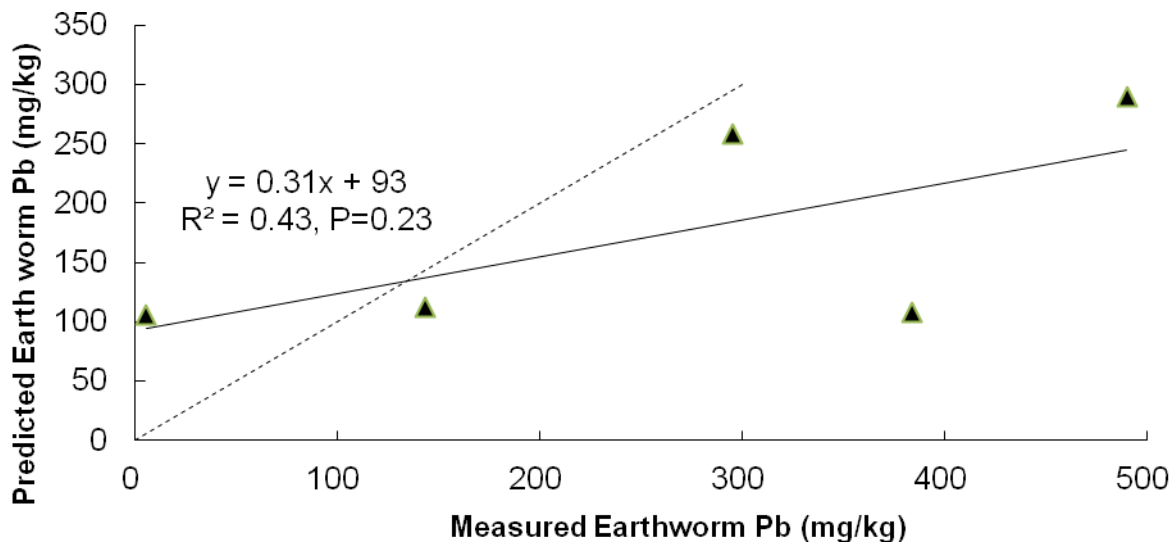
Figure B-13. Relationship between predicted and measured Pb concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model:    $\log Pb_{worm} = 0.343$    $\log Pb_{0.5\ M\ Ca(NO3)2\text{-extractable}} + 1.4$. Dotted line represents 1:1 predicted:measured.

A regression model applied to the data in Figure B-14 relating log Zn concentrations in earthworms to log 0.5 M $Ca(NO_3)_2$-extractable Zn ($R^2 - 0.08$) was not significant ($P = 0.21$). These soils were all amended with the same concentration of Zn, 300 mg/kg, and the mean ($\pm 95\%$ CL) Zn concentration in earthworms was 145 ($\pm 7.1$) mg/kg. The mean ($\pm 95\%$ CL) Zn concentration in earthworms exposed to ESTCP soil (Table B-3) was 183 ($\pm 23.2$) mg/kg. Although these means are statistically different, they are not expected to cause biological effects as the mean Zn concentration in earthworms exposed to Webster reference soil was 179 mg/kg.
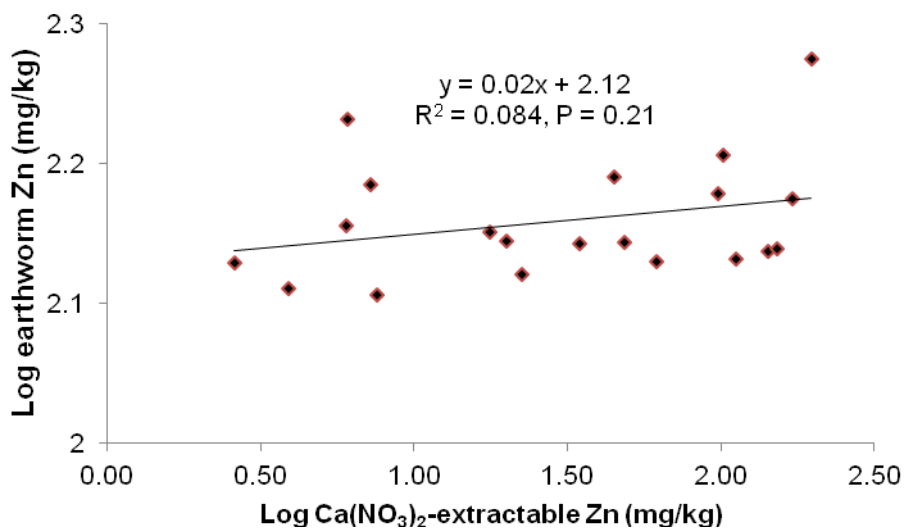
Figure B-14. Relationship between log Zn concentrations in earthworms and log 0.5 M Ca(NO$_3$)$_2$-extractable Zn. Model was developed using 22 soils differing in physicochemical characteristics amended with 300 mg/kg Zn.
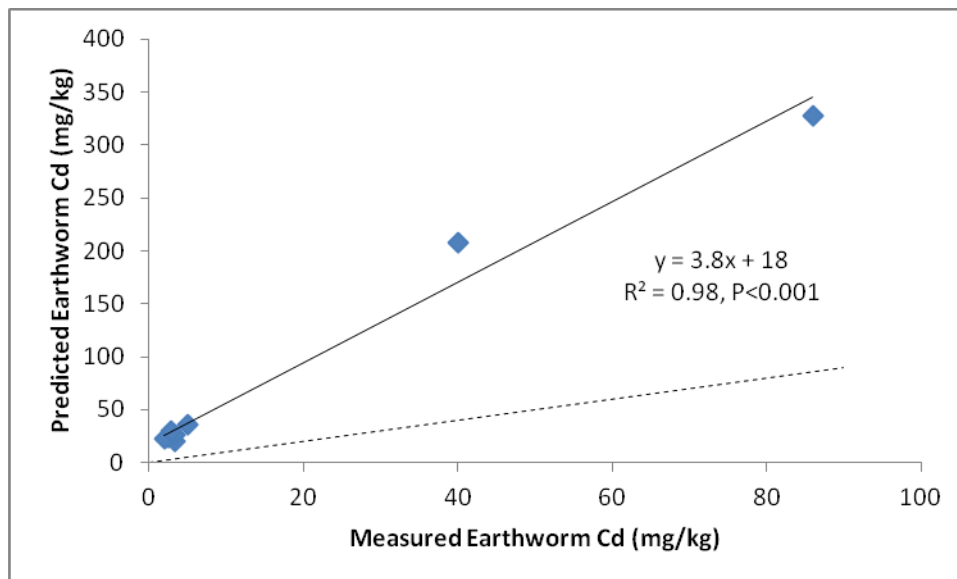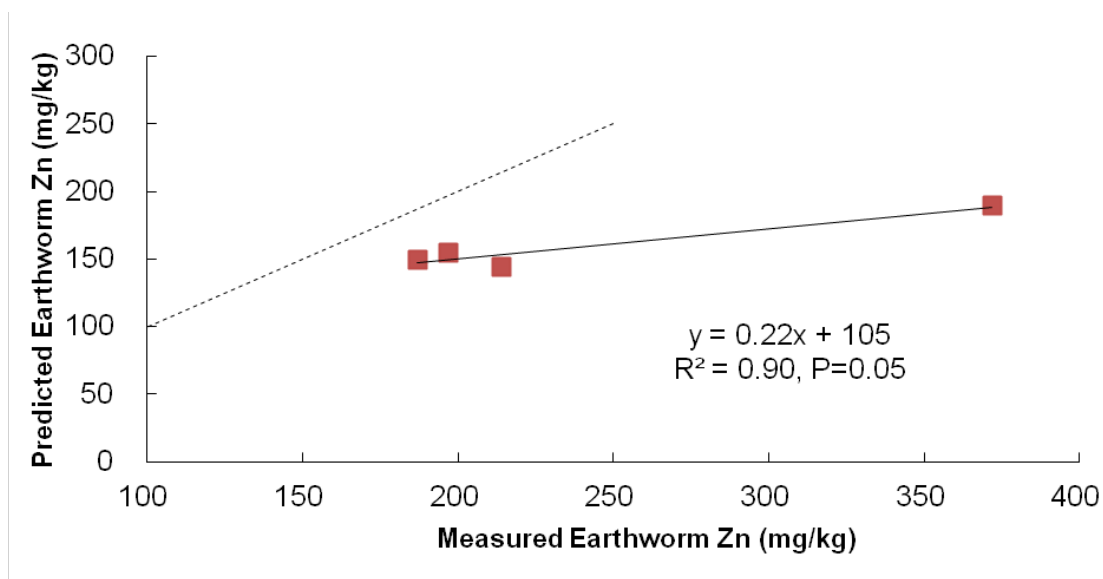


Figure B-15. Relationship between predicted and measured Pb concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model:   log Zn$_{worm}$ = 0.02 log Zn$_{0.5 \text{ M Ca(NO3)2-extractable}}$ + 2.1. Dotted line represents 1:1 predicted:measured.
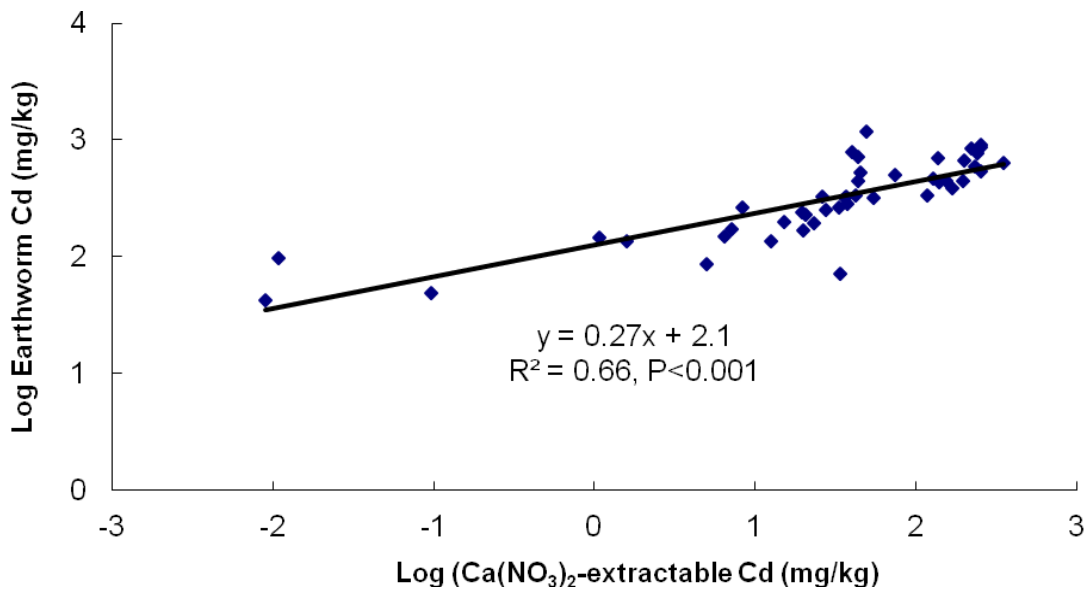
Figure B-16. Relationship between log Cd concentrations in earthworms and log 0.5 M Ca(NO$_3$)$_2$-extractable Cd. Model was developed using 27 soils differing in physicochemical characteristics and Cd amendment levels (nominal amended levels: 10, 50, or 300 mg/kg Cd).

The model in Figure B-16 relating log Cd concentrations in earthworms to log 0.5 M Ca(NO$_3$)$_2$-extractable Cd ($R^2$ – 0.66) was based upon 27 soils differing in physicochemical characteristics and amended with three different levels of Cd, either 10, 50, or 300 mg/kg Cd (nominal). Since there were only two ESTCP soils for which Ca(NO$_3$)$_2$-extractable Cd was detectable (Cherry Point and McCllelan) a correlation analysis could not be conducted. Measured Cd concentrations in worms exposed to Cherry Point and McCllelan soils were 40 and 86 mg/kg, respectively and the model over-predicted Cd bioaccumulation. The model predicted Cd concentrations in earthworms of 271 and 266 mg Cd/kg for worms exposed to Cherry Point and McCllelan soils, respectively.

**Discussion**

Soil composition was the dominant factor affecting reproduction in *E. andrei*, with hatchling production significantly lower in 95% of the ESTCP soils (reference and contaminated) compared to the Webster reference soil, but did not markedly affect adult earthworm survival. Reproduction tests conducted with *E. crypticus* and *F. candida* were less affected by soil composition with 57% and 33% of the tests, respectively, showing significantly lower reproduction than test organisms exposed to the Webster reference soil. All reproduction and survival tests conducted with Webster reference soil met validation criteria for the respective tests, suggesting healthy test organisms were used in all tests. Since ESTCP test soil composition was a variable influencing the outcome of the tests, it would be reasonable to compare test organism responses to their respective site reference soils if responses in both soils were lower than in the Webster lab reference soil. For tests conducted with *E. andrei*, in only two cases, survival in Deseret soil and reproduction in Travis soil, were earthworm responses significantly lower in contaminated compared to site reference soils (Table B-3). Arsenic appeared to be the only element in Deseret soil that was elevated relative to its site reference soil, but As levels were still lower than in Hilo (660 mg As/kg) and Pearl (619 mg As/kg) soils, where no significant mortality was observed. Whole-body residues of As were similar in worms exposed to contaminated Deseret, Hilo, and Pearl soils and were in a range that was not associated with mortality in previous studies (Lanno and Basta 2003). It would appear that increased mortality observed in worms exposed to contaminated Deseret soil was not due directly to the effects of As levels in the soil. Similarly, in Travis soil, Pb appeared to be the only metal that was elevated to a range where toxicity might occur, and decreased reproduction in earthworms exposed to contaminated Travis soil would not seem to be caused by elevated Pb concentrations alone. In

tests with *En. crypticus*, survival and reproduction were lower in contaminated Mechanicsburg

and Portsmouth soils relative to their site reference soils, and reproduction was reduced in Pearl

soil relative to its reference soil. Metal levels in soil do not explain potworm responses in

contaminated Mechanicsburg soil as only Pb levels are significantly higher than the site

reference (Table B-2), but do not fall in a range that would be toxic to potworms. Potworms

exposed to contaminated Mechanicsburg soil accumulated significantly higher levels of As

relative to potworms exposed to the site reference (Table B-4), but these levels were much lower

than in potworms exposed to other ESTCP soils where no effects on survival or reproduction

were observed, so As is not a likely cause of the observed effects (Karjalainen et al. 2009).

Contaminated Portsmouth soil was particularly toxic to *En. crypticus*, with Pb and Zn levels

significantly higher than in site reference soil. Together with potworms exposed to Pearl soil, Zn

concentrations in potworms exposed to Portsmouth were the highest of all the ESTCP soils

tested. Reproduction in potworms exposed to Pearl soil was also reduced significantly relative to

the site reference soil (Table B-4). In contrast to Portsmouth soil, contaminated Pearl soil not

only had elevated levels of Pb and Zn, but also Cu and As relative to the site reference soil.

However, the toxicity was much less than observed in Portsmouth. Responses of collembola (*F. candida*) were affected much less by the physicochemical properties of ESTCP soils (Table B-5),

as would be expected since collembolans are in contact much less with soil than oligochaetes and

dermal absorption of metals is greatly reduced. General trends suggest that for collembola

exposed to Mechanicsburg, Travis, and ORNL soils, Pb was the only element with elevated

concentrations relative to site reference soils and may be responsible for the observed effects. In

addition to elevated Pb concentrations, As and Zn were also elevated in Hilo soil relative to site

reference soil. In collembola exposed to Cherry Point and McCllelan soils, Cr levels were

elevated relative to site reference soils and may be responsible for the observed effects. Similarly, As appears to be responsible for observed effects in Concord soil.

Comparing total metal levels in all the ESTCP soils reveals that some exceeded their respective US EPA EcoSSLs (see Table B-2 for EcoSSL values). EcoSSLs are total contaminant concentrations in soil that are protective of ecological receptors that commonly come into contact with soil or ingest biota that live in or on soil. These are very conservative values that consider bioavailability and can be used to identify those contaminants of potential concern in soils requiring further evaluation in a baseline ecological risk assessment and are not soil quality standards. Although there was observed toxicity in at least one test species in all soils in which total metal levels exceeded EcoSSLs, toxicity was also observed in soils (Mechanicsburg, Deseret, Concord, ORNL) where no metal levels exceeded EcoSSLs. This may be attributable to toxicity resulting from metals with no available EcoSSL (e.g., As in Deseret), extremes in soil physical or chemical parameters (e.g., pH in ORNL), or other physical or chemical factors in the soils. EcoSSLs don't appear to provide much insight for assessing the potential toxicity of the DoD soils.

A survey of the literature did not reveal any regression-type models relating earthworm toxicity endpoints, such as mortality and reproduction, to total soil metal concentrations and soil properties. In the ESTCP studies, little mortality was observed, but decreased reproduction was observed in almost all the ESTCP soils and could be related to either the effects of soil matrix or the effects of metal contamination. Soil properties, particularly reduced organic carbon and pH, as well as texture extremes, have been shown to negatively impact the reproduction of oligochaetes during soil toxicity tests (Kuperman et al. 2006; Chelinho et al. 2011). ESTCP soils with low organic carbon content (<1.5%) and extreme textures (>50% sand) were

apparently not suitable for *E. andrei* reproduction even though no metal contamination existed and organisms were fed during bioassays, resulting in poor reproduction even in many uncontaminated reference soils. Compared to the corresponding reference soils, reproduction was significantly lower in only two contaminated soils, which could possibly be related to the metal toxicity. However, for the cases where reproduction rates in site reference soils were below validation limits (Environment Canada 2004), comparisons with responses in matched metal-contaminated soil would still be necessary and useful for the interpretation of potential toxic effects of metals. Feral *E. andrei* do not naturally inhabit soils low in organic matter and the relevance of data generated from *E. andrei* bioassays for ecological risk assessment should be used with caution, but few other standardized tests for soil-dwelling organisms exist as alternatives. The relevance of *E. andrei* as a model organism may be better understood if tests on the same soils with other indigenous earthworm species provided similar results. Other soil invertebrates that could live and reproduce in the specific soil texture may also be an alternative. For soils where no other species can be tested, earthworms may still be a possible choice since robust methods and standard protocols exists for earthworm bioassays, but additional information is needed on the effects of soil physicochemical parameters on reproduction.

The Terrestrial Biotic Ligand Model (TBLM) predicts the toxicity of metals in soils based upon estimating the free metal ion species in soil using a speciation model (WHAM VI; Tipping 1998) and relating that to binding of the metal to a biotic ligand resulting in observed toxicity (Thakali et al. 2006). To date, TBLM models exist only for Cu and Ni, limiting any application to the ESTCP data set since the TBLM is not designed to exam the toxicity of metal mixtures. Additionally, in soils where Cu and Ni levels were above their respective EcoSSL values, no decrease in *Eisenia* reproduction was observed relative to the reference soils.

Earthworm metal bioaccumulation models are usually developed from laboratory bioassays conducted with a specific earthworm species and a set of soils with a limited range of soil parameters. In order to be widely applied in ecological risk assessment, these models need to be validated with different data sets, especially if the model is to be applicable to field-contaminated soils. In this report, we examined the application of literature-based earthworm metal bioaccumulation models using data from bioassays conducted with metal-contaminated ESTCP field soils.

The predictive capacity of earthworm bioaccumulation models in the literature relating tissue metal concentrations to total soil metal concentrations and soil properties differs among metals. Few studies have examined the bioaccumulation of As, Cr, and Ni in earthworms and there are few models predicting tissue concentrations of these metals in relation to soil properties. However, among these few models, good relationships between measured metal levels in earthworms exposed to ESTCP soils and metal levels predicted by the models were found ($R^2$ range – 0.73-0.90), all using total soil concentration as the only independent variable (Figures B-1-B-3). Acceptable relationships between predicted and measured As (RMSE = 24.2%) and Cr (RMSE = 13.6%) were observed (Table B-7). The correlation observed between measured and predicted Cr levels in earthworms was contrary to that of Sample et al. (1998) which indicated that Cr concentrations in earthworms were poorly predicted by total soil Cr concentrations. Chromium concentrations predicted by the best fitting model ($R^2$ – 0.73) (Peijnenburg et al. 1999a) under-predicted Cr concentrations measured in earthworms exposed to ESTCP soils (Figure B-2, Table B-7). The bioaccumulation of Cr is highly dependent on chemical species, with Cr(VI) being more bioavailable than Cr(III) (Eisler 1986). Therefore, the difference between predicted and measured earthworm Cr concentrations may be related to the differences

in Cr speciation between soils used to develop the model and ESTCP soils. The available data for Ni in the literature were contradictory, indicating either positive, negative, or no correlation between total soil Ni and worm Ni concentrations (Neuhauser et al. 1995; Abdul Rada and Bouché 1995; Beyer et al. 1982; Sample et al. 1998). Also, the best fitting model for Ni in our study ($R^2$ – 0.88; Neuhauser et al. 1995; RMSE = 689%) grossly over-predicted earthworm Ni concentrations (Figure B-3; Table B-7), likely due to the narrow range of soil Ni from which the model was developed and extrapolation beyond Ni concentrations used in model development for the ESTCP soils.

Several studies focused on the bioaccumulation of Cd and Pb in earthworms, which provided a substantial number of models for comparison. For Cd, tissue concentration was best predicted by a model comprising total soil Cd, OM, and soil pH as the independent variables (Figure B-4), while for Pb, tissue concentrations were best predicted by a model using total soil Pb and soil pH as the independent variables (Figure B-6). Both Cd (RMSE = 106%) and Pb (RMSE = 106%) over-predicted metal levels in earthworms. Negative coefficients associated with these soil parameters indicated that increases in these parameters would decrease metal bioaccumulation in earthworms. This was consistent with the findings of several studies (Beyer et al. 1987; Janssen et al. 1997; Ma 1982; Morgan and Morgan 1988; Peijnenburg et al. 1999a; Peramaki et al. 1992). Cadmium and Pb are non-essential metals with little regulation of uptake by earthworms (Dallinger 1993; Van Gestel et al. 1993). Total soil metal concentration combined with soil properties accounted for greater than 90% of the variability in earthworm tissue burdens of these metals.

Although several bioaccumulation models existed for Cu and Zn, predictive capabilities were poor. The best model (Morgan and Morgan 1988) for Cu bioaccumulation explained about

45% of the variability in earthworm tissue burdens using total soil Cu as the independent variable (Figure B-7), while for Zn, about 62% of the variability in earthworm tissue burdens could be explained by total soil Zn concentrations (Peijnenburg et al. 1999a). However, this model over-predicted worm Zn concentrations by two to three orders of magnitude (RMSE = 590%; Figure B-8). Adding soil property parameters to the bioaccumulation models did not significantly improve the prediction. Therefore, other factors besides total soil metal concentration and soil properties affected the bioaccumulation of these metals. Both Cu and Zn are essential metals, and their uptake can be regulated by earthworms (Morgan and Morgan 1988; Van Gestel et al. 1993). Evidence of the regulation of Cu and Zn uptake was observed in earthworms exposed to the ESTCP soil since over a broad range of soil concentrations (30 to 1,889 mg/kg for Zn (63-fold); 1.0 to 423 mg/kg for Cu (423-fold)), tissue concentrations increased only slightly and remained within a range of 3.3 to 3.6-fold (102 to 372 mg/kg for Zn; 19 to 64 mg/kg for Cu), for Zn and Cu, respectively. The ability to regulate tissue levels of essential metals such as Cu and Zn explains why total soil metal concentrations combined with soil properties could not provide a reasonable prediction of earthworm tissue concentrations of Cu and Zn, especially if no toxicity was observed. Models predicting Cu and Zn levels in earthworms over the range of bioavailable soil metal concentrations that are not toxic to earthworms provide little useful information for risk assessment. The development of models that consider toxicity above the threshold of bioavailable essential metals (e.g., TBLM for Cu; Thakali et al. 2006) are required for assessing the risks of these metals to invertebrates in soils.

Another parameter often used to describe metal bioaccumulation is the bioaccumulation factor (BAF) which is the ratio of the concentration of metal in an organism to the concentration in the soil, at steady state with respect to the accumulation kinetics of the metal. Peijnenburg et al.

(1999b) developed models relating BAFs of Cd, Pb, and Zn in *En. crypticus* to soil physicochemical properties, and the application of these models to predicting BAF in *En. crypticus* exposed to ESTCP soils provided no correlations for Pb and Zn, but provided an acceptable prediction for Cd, based upon six soils (RMSE = 21%) (Figures B-9-B-11). Poor correlations for Pb and Zn may be due to the fact that assumptions regarding the independence of BAF with exposure concentration do not hold true for metals as they do with organic compounds that bioaccumulate and an inverse relationship had been observed between BAF and exposure concentration for both essential metals and non-essential metals (McGeer et al. 2003).

Models for predicting metal bioaccumulation in earthworms that incorporate total metal concentrations attempt to account for differences in the bioavailability of metals by using varying physicochemical characteristics as additional explanatory variables (Bradham et al. 2006). Another method of estimating the bioavailable fraction of metals in soils is to measure metal concentrations in aqueous extracts. Solutions ranging from distilled water to salt extracts of varying molarities have been used to estimate bioavailable metal concentrations (Lanno and Basta 2003). In an attempt to incorporate measures of the bioavailable fraction of metals in soils into a predictive model, models were developed relating 0.5 M $Ca(NO_3)_2$-extractable Cd, Pb, and Zn to earthworm metal residues (Lanno and Basta 2003; Figures B-12, B-14, B-16). However, for Cd and Pb, these models did not provide a better relationship between predicted and measured earthworm concentrations of Cd (RMSE – 111%) and Pb (RMSE – 161%) (Table B-7) than models selected from the literature that predicted earthworm metal concentrations based upon total metal levels and soil physicochemical characteristics (Figures B-4, B-6). The model relating 0.5 M $Ca(NO_3)_2$-extractable Zn to Zn concentrations in earthworms was not significant (P=0.21). These soils were all amended with the same concentration of Zn, 300

mg/kg, and the mean (±95% CL) Zn concentration in earthworms was 145 (±7.1) mg/kg. The mean (±95% CL) Zn concentration in earthworms exposed to ESTCP soil (Table B-3) was 183 (±23.2) mg/kg. Although these means are statistically different, they are not expected to cause biological effects as the mean Zn concentration in earthworms exposed to Webster reference soil was 179 mg/kg. In this case, the 0.5 M $Ca(NO_3)_2$-extractable Zn model provide better results and under-predicted (RMSE – 101%) literature models based upon total Zn concentrations and soil physicochemical characteristics. This is not unexpected since Zn concentrations are maintained at relatively constant internal concentrations in earthworms since Zn is an essential element.

Other variables that should be included in models predicting metal bioaccumulation in earthworms is exposure time and metal uptake kinetics (Yu and Lanno, 2010). For many non-essential elements (e.g., As, Cd, Pb), bioaccumulation is time-dependent with kinetics best described using a linear regression model over short-term exposures (e.g., up to 56 days). For this reason, bioaccumulation models relating metal concentrations in organisms to total soil metal concentrations and soil physicochemical characteristics may predict bioaccumulation over a specified, short duration of exposure but their relevance to predicting life-time metal bioaccumulation in soil invertebrates is unclear. In order to develop models for Cd bioaccumulation by earthworms, Yu and Lanno (2010) examined long-term uptake of Cd by *E. andrei* over a 224-day period and were able describe uptake using a one-compartment first order kinetics (1CFOK) model. Application of this model to predicting steady-state Cd bioaccumulation by *E.andrei* in two ESTCP soils (Cherry Point and McLellan) that had total Cd concentrations similar to that used in model development (19 and 22 mg Cd/kg, respectively) resulted in a very close approximation for Cd uptake by *E. andrei* exposed to Cherry Point soil

(40 mg Cd/kg measured vs 32 mg Cd/kg predicted), but under-predicted two-fold Cd

bioaccumulation in McLellan soil (86 mg Cd/kg measured vs 36 mg Cd/kg predicted). When the

entire set of ESTCP soils was included (Figure B-5), Cd bioaccumulation in earthworms was

slightly over-predicted (RMSE – 19%). While kinetics-based models appear to hold some

promise in predicting metal bioaccumulation, very few long-term kinetics studies are currently

available for model development.

ESTCP soils were often contaminated with multiple metals and it was assumed that the

effects of individual metals were additive, meaning there was no interaction between different

metals with respect to toxicity or bioaccumulation. Application of bioaccumulation models to

ESTCP data addressed each element independently without considering metal interactions on

bioaccumulation. However, such assumptions may not always be valid since metal interactions

exist and may affect metal bioaccumulation. For example, Bey et al. (1982) observed that

although total soil Cd accounted for 87% of the variability of in Cd uptake by earthworms (82%

in this study), inclusion of Zn in the model significantly improved the model fit and accounted

for an additional 5% of variability. Increased concentrations of Zn in soil were negatively

correlated with Cd in worms, theoretically due to the competition between Zn and Cd at uptake

sites.

Species is also an important factor affecting metal bioaccumulation by earthworms, with

several studies suggesting that endogeic species feeding on soil often accumulate higher metal

concentrations than epigeic species feeding on surface organic litter (Langdon et al. 2005;

Spurgeon and Hopkin 1996; Morgan and Morgan 1992, 1993, 1999; Dai et al. 2004;  Beyer et al.

1987). Some studies have developed different sets of models for different species (Laszczyca et

al. 2004; Morgan and Morgan 1988; van Vliet et al. 2005; Wright and Stringer 1980). Corp and

Morgan (1991) even differentiated models between native and introduced *Lumbricus rubellus*. In ESTCP tests, *E. andrei*, a robust species that can easily be cultured in large quantities in the laboratory, was used as the test organism. *E. andrei* has a higher reproductive rate and a shorter generation time than other species, and is responsive to a wide range of contaminants. However, the choice of *E. andrei* in toxicity and accumulation studies has been a source of criticism, principally because it is not naturally a soil-dwelling species but inhabits environments rich in organic matter such as compost piles. Additionally, since it is an epigeic species (Bouché 1972), some studies have found that *E. andrei* was less sensitive to contaminants than other species (Langdon et al. 2005; Spurgeon and Weeks 1998). For some metals, the results of our study were consistent with such findings. For example, Figures B-4 and B-6 showed that earthworm Cd and Pb concentrations in *E. andrei* were much lower than worm Cd and Pb concentrations predicted by models developed from *Lumbricus rubellus* (Ma et al. 1983; Corp and Morgan 1991). Therefore, in order to apply models developed from a specific species to another species, interspecies transfer coefficients may need to be developed.

Table B-7. Summary of the prediction of metal boaccumulation by earthworms (*Eisenia fetida*) using soil property or soil extraction data.

| Approach | Metal | Model | Summary and ability to predict metal body burdens |
|---|---|---|---|
| Soil properties | As | $\ln As_{ew}=0.9884*\ln As_s - 1.747$<br>Sample et al. 1998 | Based on total As levels; $R^2=0.90$; under predicts 0.8-16-fold, most soils 0.8-3.3 fold; RMSE = 24.2% |
| | Cd | $\ln Cd_{ew} = 6.018 + 0.787 * \ln Cd_s - 0.106 *OM - 0.402 * pH$<br>Ma et al. 1983 | Based on total Cd, organic matter, pH; $R^2=0.98$; over predicts 3.8-11.3-fold; only eight data points above DL; RMSE = 106% |
| | Cr | $\log Cr_{ew}=0.69*\log Cr_s -1.05$<br>Peijnenburg et al. 1999a | Based on total Cr; $R^2=0.73$; under predicts 0.8-7.4-fold; RMSE == 13.6% |
| | Cu | $\log Cu_{ew}=0.435*\log Cu_s +0.39$<br>Morgan and Morgan 1988 | Based on total Cu; $R^2=0.45$; under predicts 1.3-5.2-fold; RMSE = 24.7% |
| | Ni | $\log Ni_{ew}=0.98*\log Ni_s +0.67$<br>Neuhauser et al. 1995 | Based on total Ni; $R^2=0.88$; over predicts 11-95-fold; RMSE = 689% |
| | Pb | $\log Pb_{ew} = 2.65+0.897 *\log Pb_s -3.56*\log pH$<br>Corp and Morgan 1991 | Based on total Pb and pH; $R^2=0.95$; over predicts 0.5-25-fold; RMSE = 272% |
| | Zn | $\log Zn_{ew}=1.45*\log Zn_s +0.42$<br>Peijnenburg et al. 1999a | Based on total Zn; $R^2=0.62$; under predicts 1.3-5.2-fold; RMSE = 590% |
| | Cd | $C_w = 9.32 *e^{-0.008*28} + Cd_s *0.052/0.008*(1-e^{-0.008*28})$<br>Yu and Lanno 2010 | Based on Cherry Point and McLellan soils where total Cd is same as model concentration, one prediction is the same as observed and one is 2-fold higher; with all 8 data points –RMSE = 19% |
| Calcium Nitrate Extraction | Cd | $\log Cd_{ew} = 0.27*\log Cd_{Ca(NO3)2} + 2.1$<br>$R^2 = 0.66,$ | Only two soils – Cherry Point, McLellan – with total extractable Cd levels; over predicted earthworm Cd 3-6.8-fold; RMSE = 111% |
| | Pb | $\log Pb_{ew} = 0.32 Pb_{Ca(NO3)2} + 97$<br>$R^2 = 0.39, P=0.008$ | Only five soils with extractable Pb; over predicted 1.1-3.6-fold; RMSE = 161% |
| | Zn | $\log Zn_{ew} = 0.02 Zn_{Ca(NO3)2} + 2.12,$<br>$R^2=0.084, P=0.21$ | Only four soils with extractable Zn; under predicted 1.3-2-fold; RMSE = 101% |
| BAF - Soil properties *En.crypticus* | Cd | $\log BAF=1.17-0.92*\log Clay$<br>Peijnenburg et al. 1999b | Only six soils where BAF could be calculated; acceptable under-prediction; RMSE = 21% |
| | Pb | $\log BAF=0.35-0.36*pH$<br>Peijnenburg et al. 1999b | No relationship |
| | Zn | $\log BAF =3.47-0.46 *pH-0.67*\log Al_{ox}$<br>Peijnenburg et al. 1999b | No relationship |

**Conclusions**

Metal bioaccumulation and toxicity to soil invertebrates (*E. andrei, En. crypticus, F. candida*) were examined in ESTCP metal-contaminated soils (with paired reference site soils) comprising a wide range of physical and chemical characteristics and metal levels. The predictive ability of a number of different models relating soil properties to oligochaete metal bioaccumulation and toxicity as a screening tool for estimating metal bioavailability in soils was examined with the intent of validating some of these models for predicting metal bioaccumulation in soil-dwelling oligochaetes.

Key elements for predicting bioaccumulation of metals by soil invertebrates include metal concentration in the soil, soil physicochemical characteristics, and time. A review of the literature revealed many models that used total soil metal concentration as the lone predictor variable of metal bioaccumulation by soil invertebrates, and in this research some of the better models for predicting metal bioaccumulation for a few of the metals (e.g., Cr, Ni) were still those based solely upon total metal concentrations, perhaps due to the lack of many models. More recent models have attempted to include metal bioavailability in the prediction of bioaccumulation by relating soil physicochemical characteristics to metal uptake in the models. An alternative approach uses the concentration of metals in an aqueous extract of the soil as a measure of bioavailability and a predictor variable. All of the previous models do not take into consideration a basic paradigm of bioaccumulation, the kinetics of metal uptake. Very recent models have attempted to incorporate metal toxicokinetics into predictions of Cd bioaccumulation in

earthworms. In this study, we have examined the application of all these models, with varying degrees of success, to predicting the bioaccumulation of metals by earthworms from ESTCP soils. Three models (Cd, Pb, Zn) relating BAF in *En. crypticus* to soil properties were available for enchytraeids and none was found for collembola. The models can be divided into three categories: 1) Metals for which a large number of models exist in the literature (e.g., Pb, Cd); 2) Metals for which few models exist in the literature (e.g., Cr, Ni); and, 3) Essential metals (e.g., Cu, Zn).

When applying literature-based metal bioaccumulation models to assess Cd and Pb bioaccumulation by earthworms in metal-contaminated field soils, 98% of the variability in earthworm Cd concentrations could be predicted by a model comprising total soil Cd, organic matter content, and soil pH (Ma et al. 1983), while 95% of the variability in earthworm Pb concentrations could be predicted by a model including total soil Pb and soil pH (Corp and Morgan 1991). However, both these models over-predicted metal bioaccumulation (RMSE Cd – 106%; Pb – 272%) so their use in predicting bioaccumulation may be limited. A large portion of the variability in the tissue concentrations of As (90% - Sample et al. 1998), Cr (77% - Peijnenburg et al. 1999a), and Ni (88% - Neuhauser et al. 1995) could be estimated by their concentrations in soil. Even though just a few bioaccumulation models exist for these metals, the models for As (RMSE – 24.2%) and Cr (RMSE – 13.6%) provided acceptable predictions of metal uptake, while the Ni model severely over-predicted uptake (RMSE – 689%). However, for the essential metals Cu and Zn, total soil concentrations combined with soil

properties    provide a reasonable prediction of tissue concentrations for Cu (RMSE –

24.7%) but not for Zn (RMSE – 590%). These results should be viewed cautiously, since

the outcome of model prediction has little relevance since exposure concentrations in

ESTCP soils were all in the range where metal body burdens could be regulated by

worms. A model relating BAF of Cd to soil properties (Peijnenburg et al. 1999b)

provided acceptable predictions of Cd BAFs by *En. crypticus* from ESTCP soils (RMSE

– 20%) while no relationship was evident between BAFs and observed metal burdens for

Pb and Zn.

Models developed relating 0.5 M $Ca(NO_3)_2$-extractable Cd and Pb to earthworm

metal residues (Lanno and Basta 2003) did not provide a better prediction of Cd and Pb

concentrations in earthworms exposed to ESTCP soils than models selected from the

literature that predicted earthworm metal concentrations based upon total metal levels

and soil physicochemical characteristics. Models incorporating toxicokinetics of metals

were only available for Cd (Yu and Lanno, 2010) and provided reasonable estimates of

Cd concentrations in earthworms (RMSE – 19%). Suffice to say, there are no models for

a specific metal that would provide good predictions of metal bioaccumulation in all soils

and situations. For Cd and Pb, since there were many models to choose from in the

literature, it was possible to find one that provided reasonable predictions for Cd and Pb

bioaccumulation. Models that used extractable metals did not account for as much of the

observed variability but may have more general applicability to soils differing in

physicochemical characteristics, while toxicokinetic models for Cd show some promise but have only been applied in very few situations.

Metal bioaccumulation itself is only one line of evidence in an ecological risk assessment and other endpoints, such as reproduction and survival, provide important evidence of the effects of metals in soils. Reproduction and survival bioassays conducted in ESTCP soils provided ample evidence of the confounding effects of soil matrix composition on responses by the standard test organism *E. andrei*. It was also evident that the effects of soil composition were less confounding in bioassays conducted with enchytraeids or collembolan, suggesting that earthworms may not be the most suitable test organism for certain endpoints in soils of poor composition, especially with respect to organic carbon and soil texture extremes. While earthworms may be suitable for assessing metal bioaccumulation from some soils, other soil invertebrates may be more suitable for reproduction and survival endpoints. Toxicity tests exist or are being developed for soil invertebrates such as oribatid mites (Princz et al. 2010), isopods (Loureiro et al. 2005), and other invertebrates (Løkke and van Gestel, 1998) which may provide data on the toxicity of metals in soils to which earthworms are not suited.

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# Appendix B-1:    Summary of earthworm metal bioaccumulation models

| Metal | Model | $R^2$ | Reference |
|---|---|---|---|
| As | log BAF=-1.03*log Alox+1.29 | 0.0369 | Janssen et al. 1997 |
| | ln As$_{ew}$=0.9884*ln As$_s$-1.747 | 0.9045 | Sample et al. 1998 |
| Cd | log BAF=-0.43*pH+1.36*log Clay-1.39*log OM+3.19 | 0.8806 | Janssen et al. 1997 |
| | log Cd$_{ew}$=0.27*log Cd$_s$+1.4 | 0.7254 | Heikens et al. 2001 |
| | log Cd$_{ew}$=0.3*log Cd$_s$-0.3 | 0.7337 | Wright and Stringer 1980 |
| | log Cd$_{ew}$=0.3*log Cd$_s$+1.1 | 0.7337 | Corp and Morgan 1991 |
| | log Cd$_{ew}$=0.32*log Cd$_s$-0.09 | 0.7391 | Van Vliet et al. 2005 |
| | log Cd$_{ew}$=0.32*log Cd$_s$+0.33 | 0.7391 | Wright and Stringer 1980 |
| | log Cd$_{ew}$=0.39*log Cd$_s$+1.1 | 0.7572 | Heikens et al. 2001 |
| | log Cd$_{ew}$=0.45*log Cd$_s$+1.45 | 0.7718 | Spurgeon and Hopkin 1996 |
| | log Cd$_{ew}$=0.47*log Cd$_s$+1.2 | 0.7764 | Morgan and Morgan 1988 |
| | log Cd$_{ew}$=0.5*log Cd$_s$+0.7 | 0.7832 | Wright and Stringer 1980 |
| | log Cd$_{ew}$=0.51*log Cd$_s$+1.5 | 0.7854 | Neuhauser et al. 1995 |
| | log Cd$_{ew}$=0.56*log Cd$_s$+0.67 | 0.7961 | Wright and Stringer 1980 |
| | log Cd$_{ew}$=0.61*log Cd$_s$+1.3 | 0.8062 | Morgan and Morgan 1988 |
| | log Cd$_{ew}$=0.66*log Cd$_s$+1.21 | 0.8157 | Neuhauser et al. 1995 |
| | log Cd$_{ew}$=0.69*log Cd$_s$+0.4 | 0.8211 | Van Vliet et al. 2005 |
| | ln Cd$_{ew}$=0.486*ln Cd$_s$+3.740 | 0.78 | Ma et al. 1983 |
| | ln Cd$_{ew}$=0.5512*ln Cd$_s$+2.8216 | 0.7943 | Sample et al. 1998 |
| | Cd$_{ew}$=52.15*log Cd$_s$+84.37 | 0.6426 | Laszczyca et al. 2004 |
| | Cd$_{ew}$=84.91*log Cd$_s$+22.596 | 0.6426) | Laszczyca et al. 2004 |
| | log Cd$_{ew}$=1.28+0.324*log Cd$_s$-0.23*log pH | 0.7906 | Corp and Morgan 1991 |
| | log Cd$_{ew}$=1.34+0.566*log Cd$_s$-0.171*log pH | 0.8234 | Corp and Morgan 1991 |
| | ln Cd$_{ew}$=5.538+0.664*ln Cd$_s$-0.404*pH | 0.9643 | Ma et al. 1983 |
| | log Cd$_{ew}$=1.14-0.079*pH | 0.4456 | Beyer et al. 1987 |
| | log Cd$_{ew}$=1.207+0.618*log Cd$_s$-0.194*log OM | 0.8183 | Morgan and Morgan 1988 |
| | log Cd$_{ew}$=1.417+0.492*log Cd$_s$-0.181*log OM | 0.7957 | Morgan and Morgan 1988 |
| | log Cd$_{ew}$=1.93+0.480*log Cd$_s$-0.548*log OM | 0.2571 | Corp and Morgan 1991 |
| | log Cd$_{ew}$=2.04+0.209*log Cd$_s$-0.709*log OM | 0.0471 | Corp and Morgan 1991 |
| | ln Cd$_{ew}$=4.233+0.612*ln Cd$_s$-0.107*OM | 0.723 | Ma et al. 1983 |

| | | | |
|---|---|---|---|
| | $\log Cd_{ew}=2.37+0.519*\log Cd_s-0.57*\log pH-0.585*\log OM$ | 0.5376 | Corp and Morgan 1991 |
| | $\ln Cd_{ew}=6.018+0.787*\ln Cd_s-0.106*OM-0.402*pH$ | 0.9801 | Ma et al. 1983 |
| | $Cd_{ew}=713*(0.98*Cd_{soil\ pH-extactable}+0.02*Cd_{gut\ pH-extactable})-11.2$ | --- | Saxe et al. 2001 |
| Cr | $\log Cr_{ew}=0.69*\log Cr_s-1.05$ | 0.7324 | Peijnenburg et al. 1999 |
| Cu | $\log BAF=-0.65*\log Feox-0.38*\log Clay+1.38$ | 0.2177 | Janssen et al. 1997 |
| | $\log Cu_{ew}=0.01*\log Cu_s+0.23$ | 0.4198 | Wright and Stringer 1980 |
| | $\log Cu_{ew}=0.1*\log Cu_s+2.5$ | 0.4314 | Wright and Stringer 1980 |
| | $\log Cu_{ew}=0.14*\log Cu_s+1.07$ | 0.4359 | Grelle and Descamps 1998 |
| | $\log Cu_{ew}=0.15*\log Cu_s+1.2$ | 0.4369 | Heikens et al. 2001 |
| | $\log Cu_{ew}=0.229*\log Cu_s+0.726$ | 0.444 | Morgan and Morgan 1988 |
| | $\log Cu_{ew}=0.25*\log Cu_s-0.54$ | 0.4455 | Peijnenburg et al. 1999 |
| | $\log Cu_{ew}=0.291*\log Cu_s+0.944$ | 0.4481 | Corp and Morgan 1991 |
| | $\log Cu_{ew}=0.326*\log Cu_s+0.798$ | 0.4497 | Corp and Morgan 1991 |
| | $\log Cu_{ew}=0.41*\log Cu_s-0.47$ | 0.4518 | Van Vliet et al. 2005 |
| | $\log Cu_{ew}=0.435*\log Cu_s+0.39$ | 0.4519 | Morgan and Morgan 1988 |
| | $\log Cu_{ew}=0.45*\log Cu_s+0.81$ | 0.4518 | Spurgeon and Hopkin 1996 |
| | $\log Cu_{ew}=0.46*\log Cu_s+0.6$ | 0.4517 | Heikens et al. 2001 |
| | $\log Cu_{ew}=0.487*\log Cu_s+0.327$ | 0.4512 | Morgan and Morgan 1988 |
| | $\log Cu_{ew}=0.5*\log Cu_s+1.2$ | 0.4509 | Wright and Stringer 1980 |
| | $\log Cu_{ew}=0.5*\log Cu_s+1.8$ | 0.4509 | Wright and Stringer 1980 |
| | $\log Cu_{ew}=0.51*\log Cu_s-0.47$ | 0.4506 | Van Vliet et al. 2005 |
| | $\log Cu_{ew}=0.53*\log Cu_s-0.34$ | 0.4499 | Van Vliet et al. 2005 |
| | $\log Cu_{ew}=0.57*\log Cu_s+0.39$ | 0.448 | Neuhauser et al. 1995 |
| | $\log Cu_{ew}=0.67*\log Cu_s+0.35$ | 0.4404 | Neuhauser et al. 1995 |
| | $\ln Cu_{ew}=0.2414*\ln Cu_s+1.8059$ | 0.4449 | Sample et al. 1998 |
| | $Cu_{ew}=1.52*\log Cu_s+5.35$ | 0.4148 | Laszczyca et al. 2004 |
| | $Cu_{ew}=34.8*\log Cu_s-44.6$ | 0.4442 | Kennette et al. 2002 |
| | $Cu_{ew}=14.88+0.344*Cu_s$ | 0.392 | Ma et al. 1983 |
| | $Cu_{ew}=18.43+0.340*Cu_s-0.738*pH$ | 0.3949 | Ma et al. 1983 |
| | $\log Cu_{ew}=0.895+0.308*\log Cu_s-0.0561*\log OM$ | 0.4134 | Corp and Morgan 1991 |
| | $\log Cu_{ew}=1.48+0.194*\log Cu_s-0.310*\log OM$ | 0.0001 | Corp and Morgan 1991 |
| | $Cu_{ew}=21.56+0.349*Cu_s-1.272*OM$ | 0.3778 | Ma et al. 1983 |
| | $Cu_{ew}=20.57+0.350*Cu_s-1.307*OM+0.238*pH$ | 0.3759 | Ma et al. 1983 |
| | $Cu_{ew}=24.9*(1.25*Cu_{soil\ pH-extactable}-0.25*Cu_{gut\ pH-extactable})+11.9$ | --- | Saxe et al. 2001 |
| Ni | $\log BAF=-0.70*\log Alox+0.47$ | 0.203 | Janssen et al. 1997 |
| | $\log Ni_{ew}=0.98*\log Ni_s+0.67$ | 0.8827 | Neuhauser et al. 1995 |
| | $\ln Ni_{ew}=2.862+0.2074*pH$ | 0.0942 | Sample et al. 1998 |
| Pb | $\log BAF=-0.61-0.74*\log clay$ | 0.0142 | Peijnenburg et al. 1999 |
| | $\log BAF=-0.78*\log clay-0.45*\log Feox+0.46$ | 0.1199 | Janssen et al. 1997 |
| | $\log Pb_{ew}=0.22*\log Pb_s+0.64$ | 0.6107 | Spurgeon and Hopkin 1996 |

| | Equation | $r^2$ | Reference |
|---|---|---|---|
| | $\log Pb_{ew}=0.5*\log Pb_s-0.1$ | 0.6156 | Wright and Stringer 1980 |
| | $\log Pb_{ew}=0.5*\log Pb_s-1.1$ | 0.6156 | Wright and Stringer 1980 |
| | $\log Pb_{ew}=0.6*\log Pb_s-0.3$ | 0.6079 | Heikens et al. 2001 |
| | $\log Pb_{ew}=0.6*\log Pb_s+0.8$ | 0.6079 | Corp and Morgan 1991 |
| | $\log Pb_{ew}=0.61*\log Pb_s+0.02$ | 0.6069 | Neuhauser et al. 1995 |
| | $\log Pb_{ew}=0.69*\log Pb_s+0.096$ | 0.5982 | Corp and Morgan 1991 |
| | $\log Pb_{ew}=0.74*\log Pb_s+0.05$ | 0.592 | Neuhauser et al. 1995 |
| | $\log Pb_{ew}=0.8*\log Pb_s-0.5$ | 0.584 | Grelle and Descamps 1998 |
| | $\log Pb_{ew}=0.9*\log Pb_s-0.23$ | 0.5696 | Morgan and Morgan 1988 |
| | $\log Pb_{ew}=0.9*\log Pb_s-0.8$ | 0.5696 | Wright and Stringer 1980 |
| | $\log Pb_{ew}=0.9*\log Pb_s-1.1$ | 0.5696 | Wright and Stringer 1980 |
| | $\log Pb_{ew}=1.04*\log Pb_s-1.073$ | 0.5483 | Morgan and Morgan 1988 |
| | $\log Pb_{ew}=1.47*\log Pb_s-1.66$ | 0.4828 | Van Vliet et al. 2005 |
| | $\log Pb_{ew}=1.50*\log Pb_s-0.83$ | 0.4785 | Van Vliet et al. 2005 |
| | $\ln Pb_{ew}=0.7612*\ln Pb_s+0.0752$ | 0.5892 | Sample et al. 1998 |
| | $\ln Pb_{ew}=0.999*\ln Pb_s+0.525$ | 0.5547 | Ma et al. 1983 |
| | $Pb_{ew}=18.4*\log Pb_s-27$ | 0.529 | Kennette et al. 2002 |
| | $Pb_{ew}=31.008*\log Pb_s-69.097$ | 0.1958 | Laszczyca et al. 2004 |
| | $Pb_{ew}=74.43*\log Pb_s-167.9$ | 0.2139 | Laszczyca et al. 2004 |
| | $\log Pb_{ew}=1.24+0.830*\log Pb_s-2.12*\log pH$ | 0.8897 | Corp and Morgan 1991 |
| | $\log Pb_{ew}=2.140+1.720*\log Pb_s-7.097*\log pH$ | 0.8412 | Morgan and Morgan 1988 |
| | $\log Pb_{ew}=2.173+1.518*\log Pb_s-5.678*\log pH$ | 0.923 | Morgan and Morgan 1988 |
| | $\log Pb_{ew}=2.65+0.897*\log Pb_s-3.56*\log pH$ | 0.9474 | Corp and Morgan 1991 |
| | $\ln Pb_{ew}=4.355+1.056*\ln Pb_s-0.925*pH$ | 0.9113 | Ma et al. 1983 |
| | $\ln Pb_{ew}=5.233+0.7253*\ln Pb_s-0.82195*pH$ | 0.8432 | Sample et al. 1998 |
| | $\ln Pb_{ew}=1.261+1.146*\ln Pb_s-0.297*OM$ | 0.4005 | Ma et al. 1983 |
| | $\log Pb_{ew}=3.690+1.660*\log Pb_s-6.878*\log pH-1.010*\log CEC$ | 0.6538 | Morgan and Morgan 1988 |
| | $\log Pb_{ew}=4.154+1.530*\log Pb_s-6.657*\log pH-0.898*\log CEC$ | 0.6582 | Morgan and Morgan 1988 |
| | $\ln Pb_{ew}=4.157+1.113*\ln Pb_s-0.167*OM-0.746*pH$ | 0.6941 | Ma et al. 1983 |
| | $Pb_{ew}{}^{1/2}=-0.502*pH-0.064*OC-0.429*FEAL-0.177*CEC$ | --- | Bradham et al. 2006 |
| | $Pb_{ew}=112*(0.97*Pb_{soil\ pH\text{-}extactable}+0.03*Pb_{gut\ pH\text{-}extactable})+10.4$ | --- | Saxe et al. 2001 |
| | $Mortality^{1/2}=-0.75*pH+0.073*OC-0.40*FEAL+0.067*CEC$ | --- | Bradham et al. 2006 |
| | $(Relative\ Reproduction)^{1/2}=0.273*pH+0.176*OC+0.402*FEAL+0.414*CEC$ | --- | Bradham et al. 2006 |
| Zn | $\log BAF=-0.39*pH+2.1$ | 0.0483 | Posthuma et al. 1998 |
| | $\log BAF=-0.39*pH-1.06*\log Alox+0.73*\log Clay+3.04$ | 0.1914 | Janssen et al. 1997 |
| | $\log Zn_{ew}=0.01*\log Zn_s+0.23$ | 0.447 | Wright and Stringer 1980 |
| | $\log Zn_{ew}=0.1*\log Zn_s+2.5$ | 0.476 | Wright and Stringer 1980 |
| | $\log Zn_{ew}=0.14*\log Zn_s+1.07$ | 0.4883 | Grelle and Descamps 1998 |
| | $\log Zn_{ew}=0.16*\log Zn_s+2.5$ | 0.4942 | Heikens et al. 2001 |
| | $\log Zn_{ew}=0.17*\log Zn_s+1.8$ | 0.4972 | Heikens et al. 2001 |

| Equation | $R^2$ | Reference |
|---|---|---|
| $\log Zn_{ew}=0.18*\log Zn_s+2.27$ | 0.5001 | Corp and Morgan 1991 |
| $\log Zn_{ew}=0.2*\log Zn_s+2.1$ | 0.5058 | Morgan and Morgan 1988 |
| $\log Zn_{ew}=0.27*\log Zn_s+2.09$ | 0.5249 | Neuhauser et al. 1995 |
| $\log Zn_{ew}=0.29*\log Zn_s+2.2$ | 0.53 | Corp and Morgan 1991 |
| $\log Zn_{ew}=0.36*\log Zn_s+0.74$ | 0.5468 | Van Vliet et al. 2005 |
| $\log Zn_{ew}=0.38*\log Zn_s+1.81$ | 0.5512 | Neuhauser et al. 1995 |
| $\log Zn_{ew}=0.49*\log Zn_s+0.52$ | 0.5729 | Van Vliet et al. 2005 |
| $\log Zn_{ew}=0.5*\log Zn_s+1.2$ | 0.5746 | Wright and Stringer 1980 |
| $\log Zn_{ew}=0.5*\log Zn_s+1.8$ | 0.5746 | Wright and Stringer 1980 |
| $\log Zn_{ew}=0.55*\log Zn_s+0.30$ | 0.5827 | Van Vliet et al. 2005 |
| $\log Zn_{ew}=0.69*\log Zn_s+0.41$ | 0.6004 | Van Vliet et al. 2005 |
| $\log Zn_{ew}=1.45*\log Zn_s+0.42$ | 0.6191 | Peijnenburg et al. 1999 |
| $\ln Zn_{ew}=0.2373*\ln Zn_s+5.0981$ | 0.5162 | Sample et al. 1998 |
| $\ln Zn_{ew}=0.241*\ln Zn_s+6.047$ | 0.5172 | Ma et al. 1983 |
| $Zn_{ew}=-188.9*\log Zn_s+1466.7$ | 0.4437 | Laszczyca et al. 2004 |
| $Zn_{ew}=151*\log Zn_s-19$ | 0.4437 | Kennette et al. 2002 |
| $Zn_{ew}=612.19*\log Zn_s-675$ | 0.4437 | Laszczyca et al. 2004 |
| $\log Zn_{ew}=1.86+0.250*\log Zn_s-0.643*\log pH$ | 0.4359 | Corp and Morgan 1991 |
| $\log Zn_{ew}=2.42+0.202*\log Zn_s-0.281*\log pH$ | 0.4762 | Corp and Morgan 1991 |
| $\ln Zn_{ew}=4.453+0.234*\ln Zn_s+0.12845*pH$ | 0.4234 | Sample et al. 1998 |
| $\ln Zn_{ew}=6.791+0.343*\ln Zn_s-0.270*pH$ | 0.3505 | Ma et al. 1983 |
| $\ln Zn_{ew}=6.056+0.313*\ln Zn_s-0.073*OM$ | 0.1313 | Ma et al. 1983 |
| $\ln Zn_{ew}=6.878+0.439*\ln Zn_s-0.088*OM-0.298*pH$ | 0.1407 | Ma et al. 1983 |
| $Zn_{ew}=7.67*(0.82*Zn_{soil\ pH-extactable}+0.18*Zn_{gut\ pH-extactable})+102$ | --- | Saxe et al. 2001 |

$M_{ew}$: concentration of metal M in the earthworm (mg/kg).

$M_s$: total concentration of metal M in the soil (mg/kg).

OM: organic matter content (%).

BAF: Bioaccumulation Factor= $M_{ew}$ / $M_s$.

Clay: clay content (%).

Feox: Fe oxyhydroxide concentration (mmol/kg).

Alox: Al oxyhydroxide concentration (mmol/kg).

CEC: cation exchange capacity (cmol/kg).

FEAL: amorphous iron and aluminum oxides (mol/kg)

$M_{soil\ pH-extactable}$: soluble metal concentration determined after 24-h batch extraction in unbuffered DI water (µg/kg).

$M_{gut\ pH-extactable}$: soluble metal concentration predicted at pH 7.0 (µg/kg).

| Reference | Metal | Species | Experiment Type | Parameter Range |
|---|---|---|---|---|
| Beyer et al. 1987 | Cd | *Aporrectodea tuberculata* | field | |
| Bradham et al. 2006 | Pb | *Eisenia andrei* | lab | pH: 3.8-7.8<br>CEC: 3.01-32.4 cmol/kg<br>OM: 5-30%<br>FEAL: 0.009-0.195 mol/kg<br>Soil Pb: 2000 mg/kg |
| Corp and Morgan 1991 | Cd, Cu, Pb, Zn | *Lumbricus rubellus*<br>(native and introduced) | lab | pH: 3.5-8.1<br>OM: 4-35%<br>Soil Cd: 0.33-266 mg/kg<br>Soil Cu: 22-816 mg/kg<br>Soil Pb: 91-37700 mg/kg<br>Soil Zn: 416-96800 mg/kg |
| Grelle and Descamps 1998 | Cu, Pb, Zn | *Eisenia fetida* | lab | Soil Cu: 0.5-13 mg/kg<br>Soil Pb: 5-798 mg/kg<br>Soil Zn: 0.4-973 mg/kg |
| Heikens et al. 2001 | Cd, Cu, Pb, Zn | mix | field | Soil Cd: 0.1-100 mg/kg<br>Soil Cu: 10-10000 mg/kg<br>Soil Pb: 100-10000 mg/kg<br>Soil Zn: 10-10000 mg/kg |
| Janssen et al. 1997 | As, Cd, Cu, Ni, Pb, Zn | *Eisenia andrei* | lab | pH: 3.0-7.2<br>OM: 2-21.8%<br>Clay: 0.8-33.8%<br>$Fe_{ox}$: 201-16569 mg/kg<br>$Al_{ox}$: 94.4-3462 mg/kg<br>CEC: 1.7-41.8 cmol/kg<br>Soil As: 0.75-71.2 mg/kg<br>Soil Cd: 0.1-49.5 mg/kg<br>Soil Cu: 1.3-109.9 mg/kg<br>Soil Ni: 0.6-47.5 mg/kg<br>Soil Pb: 70.4-847.4 mg/kg<br>Soil Zn: 5.2-3109 mg/kg |
| Kennette et al. 2002 | Cu, Pb, Zn | *Lumbricus terrestris* | lab | Soil Cu: 23.5-2890 mg/kg<br>Soil Pb: 33.6-7110 mg/kg<br>Soil Zn: 40.1-14600 mg/kg |
| Laszczyca et al. 2004 | Cd, Cu, Pb, Zn | *Eisenia fetida*<br>*Lumbricus terrestris* | field | Soil Cd: 0.84-82 mg/kg<br>Soil Cu: 10.7-47 mg/kg<br>Soil Pb: 136-2635 mg/kg<br>Soil Zn: 151-10154 mg/kg |
| Ma et al. 1983 | Cd, Cu, Pb, Zn | *Lumbricus rubellus* | field | pH: 3.5-6.1 |

| | | | | OM: 2.2-8.6% |
|---|---|---|---|---|
| | | | | Soil Cd: 0.1-5.7 mg/kg |
| | | | | Soil Cu: 1-130 mg/kg |
| | | | | Soil Pb: 14-430 mg/kg |
| | | | | Soil Zn: 10-1220 mg/kg |
| Morgan and Morgan 1988 | Cd, Cu, Pb, Zn | *Lumbricus rubellus* <br> *Dendrobaena veneta* | field | Soil Cd: 0.1-350 mg/kg <br> Soil Cu: 26-2740 mg/kg <br> Soil Pb: 170-24600 mg/kg <br> Soil Zn: 160-45000 mg/kg |
| Neuhauser et al. 1995 | Cd, Cu, Ni, Pb, Zn | mix | field | Soil Cd: 0.01-1000 mg/kg <br> Soil Cu: 0.1-1000 mg/kg <br> Soil Ni: 1.25-2.4 mg/kg <br> Soil Pb: 0.01-100000 mg/kg <br> Soil Zn: 10-100000 mg/kg |
| Peijnenburg et al. 1999 | Cr, Cu, Pb, Zn | *Eisenia andrei* | lab | Soil Cr: 3.2-987.9 mg/kg <br> Soil Cu: 1.1-108 mg/kg <br> Soil Pb: 3.5-849.5 mg/kg <br> Soil Zn: 5.3-3138.7 mg/kg |
| Posthuma et al. 1998 | Zn | *Eisenia fetida* | lab | Soil Zn: 52.3-3112.6 mg/kg |
| Sample et al. 1998 | As, Cd, Cu, Ni, Pb, Zn | mix | field | pH: 2.8-9.96 <br> Soil As: 0.77-79.2 mg/kg <br> Soil Cd: 0.06-467 mg/kg <br> Soil Cu: 3.43-1000 mg/kg <br> Soil Ni: 11.4-57 mg/kg <br> Soil Pb: 0.79-24550 mg/kg <br> Soil Zn: 12.5-183000 mg/kg |
| Saxe et al. 2001 | Cd, Cu, Pb, Zn | *Eisenia andrei* | lab | pH: 3.52-7.9 |
| Spurgeon and Hopkin 1996 | Cd, Cu, Pb, Zn | mix | field | Soil Cd: 0.5-312 mg/kg <br> Soil Cu: 6.3-2610 mg/kg <br> Soil Pb: 56.2-15600 mg/kg <br> Soil Zn: 31.6-32900 mg/kg |
| Van Vliet et al. 2005 | Cd, Cu, Pb, Zn | *Lumbricus rubellus* <br> *Aporrectodea caliginosa* <br> *Allolobophora chlorotica* | field | Soil Cd: 2.81-4.5 mg/kg <br> Soil Cu: 69.9-95.3 mg/kg <br> Soil Pb: 186.5-269.4 mg/kg <br> Soil Zn: 555.8-784.7 mg/kg |
| Wright and Stringer 1980 | Cd, Cu, Pb, Zn | *Lumbricus terrestris* <br> *Aporrectodea caliginosa* <br> *Allolobophora chlorotica* <br> *Aporrectodea longa* <br> *Aporrectodea rosea* | field | Soil Cd: 1-10 mg/kg <br> Soil Pb: 92-147 mg/kg <br> Soil Zn: 89-617 mg/kg |

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# APPENDIX C
# PLANT PHYTOACCUMULATION STUDY

**PREDICTION OF CONTAMINANT PHYTOACCULUMATION USING SOIL PROPERTY OR SOIL EXTRACTION SOIL DATA**

Contaminant phytoaccumulation was determined from plant bioassays for soils from 12 study sites. For ecological risk estimates, metal phytoavailability was estimated from soil-property driven multiple regression models developed using bioaccumulation data from two previous study studies; SERDP ER-1210 (Dayton et al., 2006) and a study sponsored by the National Center for Environmental Assessement (NCEA, Lanno et al., 2003).   A separate approach involved the use of soil extraction methods, used to estimate metal(loid) phytoavailability, to predict contaminant phytoaccumulation.   Regression models developed using bioaccumulation data from the NCEA study were used to predict contaminant phytoaccumulation in the study soils. Comparison of the actual contaminant phytoaccumulation from bioassays with predicted toxicity from in vitro models were used to quantify the ability of in vitro models to predict actual phytoaccumulation in field DoD soils. This was the basis for validation of the soil property or soil extraction methods for field DoD soils.

**MATERIALS AND METHODS**

**Soil Contaminant Spiking and Ageing for NCEA and SERDP soils**

Uncontaminated soils were spiked with metal salts and aged to minimize the "salt effect" (Basta et al., 2005). Soils were spiked with only one metal to avoid competitive adsorption effects. Soils from the NCEA study (Table C-1) were spiked with one contaminant and to achieve one contaminant soil concentration.  NCEA soils were spike with reagent grade $Na_2HAsO_4 \cdot 7H_2O$ at 250mgAs/kg, $Cd(NO_3)_2 \cdot 4 H_2O$, at 50 mg/kg, or $Pb(NO_3)_2$ at 2000 mg Pb/kg.  Soils from the SERDP ER-1210 study (Table C-2) were spiked with one contaminant at multiple contaminant concentrations.  ER-1210 soils were spiked with reagent grade $Na_2HAsO_4 \cdot 7H_2O$ at 10, 50, 100, 200 and 300 mgAs/kg, $Cd(NO_3)_2 \cdot 4 H_2O$, at 10, 50, 100, 200 and 300 mg/kg, or $Pb(NO_3)_2$ at 250, 500, 1000, 3000 and 5000 mg Pb/kg.   One liter of spiking solution was mixed with 5.0 kg of soil.  Additional deionized water was added to form a saturated paste and was thoroughly mixed.  The spiked soils underwent 3 wet-dry cycles at 60 °C for 24 h.  Heavy metals added as a salt can result in a "salt effect" where metal availability is greater in spiked soil than non-spiked contaminated soil. The 3 wet-dry cycles reduces the salt effect by increasing the reaction between the soil matrix and metal contaminants.  Soil salinity, as measured by the electrical conductivity (EC) of a water-saturated soil paste, was measured in spiked soils to ensure that metal salt spiking had not increased salinity enough to inhibit germination.  The EC of spiked soils was determined after the second wet-dry cycle.  Soils that had EC > 1.5 dS/m were leached with deionized water until the soil EC < 1.5 dS/m.  Spiked soils that had EC < 1.5 dS/m were not leached.  The final metal/loid concentration of spiked soils after leaching was confirmed, by microwave assisted acid digestion according to U.S. EPA Method 3051 to be within 10% of the expected spike level.  Spiked soils were then aged by wetting drying the soils for 2 years to ensure complete reaction of the contaminant with the soil thereby minimizing the salt effect.

**Table C-1.** Soil properties and summary statistics of NCEA soils

| Soil | Soil Horizon | Soil Properties† | | | | | |
|------|------|------|------|------|------|------|------|
| | | FEAL | CEC | OC | AspH‡ | CdpH‡ | PbpH‡ |
| | | mol/kg | cmol/kg | % | | | |
| Canisteo | A | 0.0570 | 30.5 | 3.00 | 7.55 | 7.80 | 7.60 |
| Dennis | A | 0.0830 | 9.77 | 1.90 | 4.90 | 4.80 | 4.75 |
| Dennis | B | 0.0660 | 14.6 | 0.80 | 5.60 | 5.65 | 5.20 |
| Norge | A | 0.0450 | 4.57 | 1.20 | 4.00 | 4.00 | 3.80 |
| Hanlon | A | 0.0440 | 16.3 | 1.60 | 7.00 | 6.50 | 6.65 |
| Taloka | A | 0.0550 | 4.85 | 1.20 | 4.65 | 5.10 | 4.15 |
| Kirkland | A | 0.0610 | 14.0 | 1.45 | 5.10 | 4.80 | 4.80 |
| Luton | A | 0.0690 | 32.4 | 2.00 | 7.15 | 7.05 | 6.60 |
| Mansic | A | 0.0260 | 16.5 | 1.50 | 7.95 | 7.30 | 7.70 |
| Mansic | B | 0.0110 | 11.7 | 0.53 | 8.00 | 7.75 | 7.80 |
| Osage | A | 0.1280 | 28.3 | 2.30 | 6.00 | 6.50 | 6.0 |
| Osage | B | 0.1950 | 27.5 | 2.00 | 6.15 | 6.10 | 5.90 |
| Pond Creek | A | 0.0580 | 10.7 | 1.90 | 4.65 | 4.60 | 4.10 |
| Pond Creek | B | 0.0490 | 12.5 | 0.80 | 5.95 | 5.75 | 5.15 |
| Teller | A | 0.0300 | 3.01 | 0.85 | 4.30 | 3.90 | 4.30 |
| Pratt | A | 0.0100 | 4.40 | 0.90 | 6.30 | 5.50 | 4.60 |
| Pratt | B | 0.0090 | 3.40 | 0.50 | 6.00 | 5.45 | 5.15 |
| Richfield | B | 0.0330 | 22.4 | 1.10 | 7.55 | 6.70 | 6.35 |
| Summit | A | 0.0890 | 29.4 | 2.40 | 7.25 | 6.95 | 6.95 |
| Summit | B | 0.0360 | 27.6 | 1.25 | 6.65 | 6.85 | 6.45 |
| SERDP | | | | | | | |
| Kirkland | A | 0.0619 | 14.2 | 1.43 | 6.27 | 6.27 | 6.27 |
| Richfield | B | 0.0470 | 27.9 | 0.657 | 7.76 | 7.76 | 7.76 |
| Teller | A | 0.0690 | 4.13 | 0.406 | 4.78 | 4.78 | 4.78 |
| Sassafras | A | 0.0530 | 4.15 | 0.721 | 5.49 | 5.49 | 5.49 |
| Webster | A | 0.0910 | 25.7 | 2.39 | 6.06 | 6.06 | 6.06 |
| | | | | | | | |
| Minimum | | 0.009 | 3.01 | 0.406 | 4.00 | 3.90 | 3.80 |
| Maximum | | 0.195 | 32.4 | 3.00 | 8.00 | 7.80 | 7.80 |
| Mean | | 0.059 | 16.0 | 1.39 | 6.12 | 5.98 | 5.77 |

† FEAL is reaction Fe and Al oxides, CEC is soil cation exchange capacity, OC is organic carbon content.
‡ The pH measured in soil spiked with As, or Cd, or Pb.

**Table C-2.** Taxonomic classifications of soils from the NCEA and SERDP projects.

| Soil Taxonomic Classification | Soil Series | Horizon |
|---|---|---|
| Fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls | Canisteo | A |
| Fine, mixed, thermic Aquic Argiudolls | Dennis | A |
| | | B |
| Loamy, mixed, active, thermic Arenic Haplustalfs | Dougherty | A |
| Coarse-loamy, mixed, superactive, mesic Cumulic Hapludolls | Hanlon | A |
| Fine, mixed, superactive, thermic Udertic Paleustolls | Kirkland | A |
| Fine, smectitic, mesic Typic Endoaquerts | Luton | A |
| Fine-loamy, mixed, superactive, thermic Aridic Calciustolls | Mansic | A |
| | | B |
| Fine-silty, mixed, active, thermic Udic Paleustolls | Norge | A |
| Fine, smectitic, thermic, Typic Epiaqerts | Osage | A |
| | | B |
| Fine-silty, mixed, superactive, thermic Pachic Argiustolls | Pond Creek | A |
| | | B |
| Sandy, mixed, mesic Lamellic Haplustalfs | Pratt | A |
| | | B |
| Fine, smectitic, mesic Aridic Argiustolls | Richfield | B |
| Fine-loamy, siliceous, semiactive, mesic Typic Hapludults | Sassafras | A |
| Fine, smectitic, thermic oxyaquic Vertic Argiudolls | Summit | A |
| | | B |
| Fine, mixed, thermic Mollic Albaqualfs | Taloka | A |
| Fine-loamy, mixed, active, thermic Udic Argiustolls | Teller | A |
| Fine-loamy, mixed, superactive, mesic Typic Endoaquolls | Webster | A |

**Determination of Soil Properties**

All analyses were performed on duplicate samples of air-dried soil (< 2 mm). Soil pH was determined in 1:1 soil: deionized water suspension using a combination pH electrode (Thomas, 1996). Because metal salt addition can cause acidification due to metal hydrolysis (Basta and Tabatabai (1992), soil pH was measured on control (unspiked) and on metal-spiked soils. Soil pH measured on metal-spiked soils was used for all statistical analyses using soil pH. Soil organic carbon (OC) was determined by oxidation of organic C by acid dichromate reduction (Heanes, 1984). Amorphous Fe and Al oxide content was determined by acid ammonium oxalate extraction (McKeague and Day, 1996) and CEC was determined using the unbuffered salt ($BaCl_2$) extraction method (Sumner and Miller, 1996). Blanks, spikes and a certified reference soil (CRM020-050, RTC Corporation, Laramie, WY, USA) were used for quality assurance and quality control in the determination of Pb levels.

**Soil Extraction Methods**

Three soil extraction methods were used. Calcium nitrate solution was used to extract cationic Cd and Pb from study soils. Soil extraction with 0.1 M $Ca(NO_3)_2$ solution at 1:20 soil:solution ratio. Soil was extracted for 16 h followed by filtration of the supernatant using 0.45 μ membrane filtration. Deionized water was used to estimate pore water As, Cd, and Pb in soils. Pore water was determined by extraction of soil with deionized water (1:1 w/w) for 4 h followed by filtration of the supernatant using 0.45 μ membrane filtration. Mehlich 3 soil extraction, commonly used to measure phytoavailable phosphate, was used to extract phytoavailable As in soil. Soil was extracted according to Mehlich (1984). In this procedure an acidic solution containing fluoride extracts soil at 1:10 soil:solution ratio for 5 min followed by filtration of the supernatant using 0.45 μ membrane filtration. All extracted metal(loids) were quantified using ICP AES.

**Plant Bioassay**

Spiked soil (800 g) was mixed with 50%, by volume, vermiculite in 1 L pots. To prevent Pb from leaching, the pots were not allowed to drain. Twenty lettuce (*Lactuca sativa var. Paris Island Cos)* seeds were planted per pot. Three replicates of each soil (Pb-spiked and control) were grown in a completely randomized design. Plants were grown in a controlled environment growth chamber with 18h of light/day, daytime temperatures of 20°C, and night temperatures of 18.5°C. To ensure that all soils had adequate nutrition, macro nutrients were tested and adjusted. Plant available phosphorus (P) and potassium (K) were determined using the Mehlich 3 (Mehlich, 1984) extraction with subsequent analysis by ICP-AES. Plant available nitrogen ($NO_3$-N and $NH_4$-N) was determined by a 1$M$ KCl extraction followed by automated flow injection analysis (Mulvaney, 1996). All soils had adequate levels of plant nutrients after fertilizer addition of Miracle Gro™ (15% N + 30% $P_2O_5$ + 15% $K_2O$). To balance nitrogen due to $NO_3$ addition as the Pb salt with soil spiking, an additional 200 mg/kg of N was applied to the control pots as $NH_4NO_3$. Percent germination was determined at 7 days. Pots were thinned to 5 plants per pot at 14 days. Lettuce was harvested after 40 days, rinsed in deionized water, and dried at 70°C for 48 h and crushed by hand. The dried material was weighed to determine dry matter growth (DMG). Dry lettuce tissue (0.25 g) was predigested for 4 h in 10 mL of nitric acid. Predigested samples were digested at 140°C for 4h, or until clear (Zarcinas et al., 1987).

Filtered (0.45 μm) solutions were analyzed for Pb by ICP-AES.  To account for differences in lettuce biological endpoints due to differences in soil quality (i.e., acidity, texture), dry matter growth (DMG) and germination (G) are presented relative to their controls.

**Statistical Analyses**
Statistical modeling was performed using two multiple regression models: multiple linear regression (MLR) and ridge regression (RR).  Both types of models were fit to the data using PROC REG in SAS 9.2.  For the MLR models, model selection was not performed; we included all five independent variables (pH, OC, FEAL, CEC, and Total) in each model.  For the RR models, an extra penalty term is added to the statistical model.  This penalty term can be tuned to adjust the parameter estimates, increasing the bias in the parameter estimates while decreasing the influence of multicollinearity on the parameter estimates.  These biased estimates produce a model that does not fit the observed data as closely as the MLR.  In all cases, the $R^2$ for the MLR will be superior to the one obtained from the RR.  However, the biased estimates produced by the RR often produce a better predictive model, and that was the central goal of our model development.

 When using the RR approach, we chose the value of the tuning parameter by selecting the value that minimizes the PRESS statistic.  The PRESS statistic is calculated by removing each observation, in turn, from the dataset; fitting the model using the remaining n – 1 observations; using the model fit to obtain a predicted value for the removed observation; and calculating the squared error of prediction for the removed observation.  After cycling through each observation in the dataset in this manner, the squared errors of prediction are summed to obtain the final PRESS statistic.  The model with the lowest PRESS statistic is declared to have the best predictive ability.  PRESS statistics cannot be compared between RR models with different dependent variables, and there isn't a specific value of the PRESS statistic that can be considered adequate for declaring a model to have good predictive ability.  However, the PRESS statistic can be used to compare two or more RR models with the same dependent variable.

Statistical models were developed using soil property and plant uptake data from a combined NCEA and SERDP ER-1210 database.  Both MLR and RR models were developed.  The developed models were evaluated to determine their ability to predict contaminant uptake for the ESTCP study soils.

**RESULTS AND DISCUSSION**

**Soil Properties**
The range in soil chemical and physical properties for the NCEA and SERDP soils is shown in Table C-1.  The amorphous Fe plus Al oxide (FEAL) content ranged from 0.009 to 0.195 mol/kg, with a mean of 0.059 mol/kg .  The coefficient of variation (CV) for amorphous Al ranged from 0.1 to 5.8% with a mean CV value of 2.8%.  The coefficient of variation (CV) for amorphous Fe ranged from 0.5 to 7.7% with a mean CV value of 3.9%.  There was a wide range in soil CEC from 3.01 to 32.4 $cmol_c$/kg with a mean of 16.0 $cmol_c$/kg.  The coefficient of variation (CV) for soil CEC ranged from 0.0 to 1.5% with a mean CV value of 0.5%.  Soil OC

ranged from 0.406 to 3.00 % with a mean of 1.39%.  The coefficient of variation (CV) for soil OC ranged from 0.0 to 12.5% with a mean CV value of 2.7%.

Spiking of soils with cationic metal salts can decrease the soil pH.  Therefore, the soil pH after spiking and ageing was used for statistical analysis using soil pH.  The pH for As-spiked soils ranged from 3.80 to 7.80 with a mean of 5.60.   The pH for Cd-spiked soils ranged from 3.90 to 7.80 with a mean of 5.98.  The pH for Pb-spiked soils ranged from 3.80 to 7.80 with a mean of 5.77 (Table C-1). The coefficient of variation (CV) for soil pH ranged from 0.0 to 1.1% with a mean CV value of 0.4%.   Comparison of soil pH of the same soil spiked with As, Cd, or Pb shows the Pb lowered pH significantly for several soils.  This consistent with acidity from hydrolysis of metal salts (i.e., Pb, Cd) because Pb was added in the greatest molar amount. Lead decreased soil pH up to 0.6 unit on unbuffered sandy soils such as Pond Creek (Table P1) but had lesser effect on highly buffered clay (i.e., Osage) or calcareous soil (i.e., Mansic).  Many studies do not measure soil pH after spiking.  This could be a significant source of error if ignored.

**Plant Phytoaccumulation of As, Cd, and Pb from study soils**
NCEA Plant Bioassay
Both lettuce and ryegrass were grown in NCEA soils.  Tissue contaminant concentration is summarized in Table C-3.  The wide range in contaminant tissue concentrations (Table C-3) resulting from a single soil spike level indicates that phytoavailability is being modified by soil properties. Plants grown in NCEA soils spiked at 250 mg As/kg had wide range in lettuce tissue As ranging from 0.76 to 31.1 mg/kg with a mean of 11.1 mg/kg and a wide range in ryegrass tissue As ranging from 4.39 to 109 mg/kg with a mean of 26.1 mg/kg.  Plants grown in NCEA soils spiked at 50 mg Cd/kg had a wide range in lettuce tissue Cd ranging from 23.8 to 128 mg/kg with a mean of 66.0 mg/kg and wide range in ryegrass tissue Cd ranging from 3.00 to 66.6 mg/kg with a mean of 27.0 mg/kg.  Plants grown in NCEA soils spiked at 2000 mg Pb/kg had lettuce tissue Pb ranging from 3.22 to 114 mg/kg with a mean of 41.6 mg/kg and ryegrass tissue Pb ranging from 16 to 236 mg/kg with a mean of 90.3 mg/kg.

SERDP Plant Bioassay
Ryegrass tissue contaminant concentration is summarized in Table C-4. Ryegrass tissue As concentration, ranged from 0.7 to 3.7 mg As/kg for soils spiked with 10 mg As/kg, from 4.20 to 13.3 mg As/kg for soils spiked with 50 mg As/kg, from 8.89 to 17.5 mg As/kg for soils spiked with 100 mg As/kg and from 16.7 to 21.9 mg As/kg for soils spiked with 200 mg As/kg. Ryegrass tissue Cd widely ranged from 3.40 to 32 mg Cd/kg for soils spiked with 10 mg Cd/kg, and from 11.4 to 91.7 mg Cd/kg for soils spiked with 50 mg Cd/kg.  Ryegrass tissue Pb widely ranged from 3.88 to 110 mg Pb/kg for soils spiked with 250 mg Pb/kg, from 6.32 to 91.2 mg Pb/kg for soils spiked with 500 mg Pb/kg, from 16.8 to 143 mg Pb/kg for soils spiked with 1000 mgPb/kg and from 114 to 167 mgPb/kg for soils spiked with 3000 mg Pb/kg.  Missing data points are due to plant death and missing soil for Richfield As (Table C-4).

ESTCP Plant Bioassay
Both lettuce and ryegrass were grown in ESTCP soils.  Data is shown for all dry matter growth (DMG) and metal(loid) tissue concentrations (Table C-5).  Metal(loid) plant uptake occurred on

uncontaminated and contaminated soils. Only plant tissue data for contaminated soils (Table C-5 bolded and underlined values) were used for statistical analyses.

Lettuce DMG ranged from 0.110 to 5.60g with a mean of 3.33g, while ryegrass tissue ranged from 1.02g to 7.75 g with a mean of 3.58g. In As contaminated soils, tissue As had a narrow range from 2.88 to 5.16 mg/kg for lettuce and < 2 to 4.64 mg/kg for ryegrass. Mean tissue As was lower for ESTCP soils (Table C-5) than mean lettuce and ryegrass As grown on NCEA (Table C-3) and SERDP soils (Table C-4). Total soil As alone cannot explain this difference because several ESTCP soils had As contents much greater than the ≤ 250 mg/kg As of the

**Table C-3.** Contaminant phytoaccumulation in plant bioassay lettuce and ryegrass tissue for NCEA soils.

| Soil Series | Horizon | Tissue Contaminant† | | | | | |
| | | Tissue As | | Tissue Cd | | Tissue Pb | |
| | | Lettuce | Ryegrass | Lettuce | Ryegrass | Lettuce | Ryegrass |
| | | mg/kg | | | | | |
| Canisteo | A | na | 16 | na | 10.6 | 8.72 | 36.6 |
| Dennis | A | 2.43 | 15 | 60.4 | 21.5 | na | 93.7 |
| Dennis | B | 0.763 | 4.39 | | 3.0 | na | 84.4 |
| Norge | A | 26.5 | 26.1 | 82.9 | 47.5 | 61.3 | 236 |
| Hanlon | A | na | 15.9 | 23.8 | 16.9 | 9.2 | 45 |
| Taloka | A | 29.7 | 26.1 | 77 | 29.1 | 37.7 | 150 |
| Kirkland | A | 14.8 | 14.8 | 52.3 | 27.9 | 50.4 | 121 |
| Luton | A | 4.58 | 19.5 | na | 18.5 | 16.5 | 31 |
| Mansic | A | na | 35.7 | na | na | 60 | 58.0 |
| Mansic | B | na | 109 | na | na | na | 64.0 |
| Osage | A | 5.16 | . | 28.1 | 13.2 | 3.22 | 16.0 |
| Osage | B | 5.6 | 4.78 | 28.9 | 15.5 | 15.3 | 25 |
| Pond Creek | A | 31.1 | 16.8 | 57.4 | 26.2 | 43.1 | 144 |
| Pond Creek | B | 9.42 | 16.1 | 75.7 | 26.2 | na | 80.3 |
| Teller | A | na | 25 | 128 | 66.6 | 108 | 186 |
| Pratt | A | na | na | na | na | na | 122 |
| Pratt | B | na | 88.9 | na | 64.8 | na | 148 |
| Richfield | B | na | 17.3 | na | na | na | 75 |
| Summit | A | 1.61 | 8.28 | na | 11.1 | na | 17 |
| Summit | B | 1.12 | 9.54 | na | 17.8 | 13.4 | 38.0 |
| | | | | | | | |
| min | | 0.763 | 4.39 | 23.8 | 3 | 3.22 | 16.0 |
| max | | 31.1 | 109 | 128 | 66.6 | 114 | 236 |
| mean | | 11.1 | 26.1 | 66.0 | 27 | 41.6 | 90.3 |

† na is data not available because of phytotoxicity

**Table C-4.** Contaminant phytoaccumulation in plant bioassay lettuce and ryegrass tissue for SERDP ER-1210 soils.

| Soil Contaminant concentration mg/kg | Tissue contaminant concentration, mg/kg, for each soil type | | | | |
|---|---|---|---|---|---|
| | Kirkland | Richfield | Teller | Sassafras | Webster |
| As | | | | | |
| 10 | 0.7 | | 3.7 | 0.96 | 1.13 |
| 50 | 5.34 | | 13.3 | 7.12 | 4.2 |
| 100 | 15.7 | | 17.5 | 16.3 | 8.89 |
| 200 | 21.9 | | na | na | 16.7 |
| Cd | | | | | |
| 10 | 5.2 | 5.6 | 32 | 18.4 | 3.4 |
| 50 | 19.4 | 16.2 | 91.7 | 91.4 | 11.4 |
| Pb | | | | | |
| 250 | 9.5 | 3.88 | 42.9 | 110 | 4.56 |
| 500 | 20.1 | 14.4 | 91.2 | 179 | 6.32 |
| 1000 | 68.2 | 49.7 | 143 | na | 16.8 |
| 3000 | 167 | 114 | na | na | na |

† na is data not available because of phytotoxicity

**Table C-5.** Dry matter growth and contaminant phytoaccumulation in plant bioassay lettuce and ryegrass tissue for ESTCP study soils.

| | | Lettuce Tissue | | | | Ryegrass Tissue | | |
|---|---|---|---|---|---|---|---|---|
| | | | Tissue | | | | | |
| Soil | DMG† | As | Cd | Pb | DMG | As | Cd | Pb |
| | g | mg/kg | | | g | mg/kg | | |
| Concord | 4.63 | **4.83‡** | 1.0 | <2 | 7.75 | 4.64 | <0.2 | <2 |
| Cherry Point | 0.110 | <2 | **29.3** | <2 | 4.49 | <2 | **5.55** | <2 |
| Deseret | 0.65 | **5.16** | 1.38 | <2 | 1.02 | **2.87** | 0.27 | <2 |
| Hill | 3.20 | <2 | **78.0** | <2 | 1.27 | <2 | **20.3** | <2 |
| Hilo | 2.58 | **3.69** | 2.17 | **7.63** | 2.84 | **2.36** | 0.27 | **6.76** |
| McCllelan | 5.05 | <2 | **29.7** | **2.00** | 4.91 | <2 | **8.65** | **<2** |
| Mechanichsburg | 2.96 | <2 | 0.26 | **8.96** | 2.59 | <2 | <0.2 | **2.10** |
| ORNL | 2.99 | <2 | 0.69 | **47.3** | 2.71 | <2 | <0.2 | **51.6** |
| Pearl City | 5.60 | **2.88** | 1.21 | **5.73** | 6.81 | **<2** | <0.2 | **<2** |
| Port | 4.39 | <2 | 1.49 | **93.8** | 1.51 | <2 | 0.99 | **262** |
| Travis | 4.45 | <2 | 0.57 | **35.6** | 3.51 | <2 | 0.38 | **76.6** |

† DMG is dry matter growth.
‡ Bolded and underlined values represent values from contaminated soils and  were used for statistical analyses.

NCEA and SERDP soils.  Thus, several ESTCP soils significantly reduced phytoavailable As.

In Cd contaminated ESTCP soils, tissue Cd concentrations ranged from 29.3 to 78.0 mg/kg for lettuce and 5.55 to 20.3 mg/kg for ryegrass.  These values are comparable to tissue Cd found in NCEA and SERDP soils.  In Pb contaminated soils, tissue Pb concentrations ranged from 2.00 to 93.8 mg/kg for lettuce and < 2 to 262 mg/kg for ryegrass.  Where tissue concentrations was below detection limit (<2 mg/kg), half of the detection limit (1 mg/kg) was used in statistical analysis.

**Soil Extractions**
Extractable contaminant data for NCEA and ESTCP soils is shown in (Table C-6).  Data was included only if there is corresponding plant tissue data.  For NCEA soils pore water extractable As ranged from 0.020 to 20.3 mg/kg with a mean of 5.56 mg/kg, while Cd ranged from 0.030 to 17.2 mg/kg with a mean of 2.72 mg/kg and Pb ranged from 0.150 to 124 mg/kg with a mean of 21.8 mg/kg.  Calcium nitrate extractable Cd ranged from 5.00 to 49.3 with a mean of 26.3, and Pb ranged from 5.11 to 1085 mg/kg with a mean of 417.  Mehlich 3 extractable As ranged from 8.64 to 164 mg/kg with a mean of 70.3 (Table C-6). For ESTCP soils pore water extractable As ranged from < 0.1 to 1.19 mg/kg with a mean of 0.518 mg/kg, while Cd ranged from < 0.1 to 0.869 mg/kg with a mean of 0.417 mg/kg and Pb ranged from 0.160 to 33.3 mg/kg with a mean of 9.88 mg/kg.  Calcium nitrate extractable Cd ranged from < 2 to 16.6 with a mean of 12.0, and Pb ranged from < 2 to 603 mg/kg with a mean of 244.  Mehlich 3 extractable As ranged from 7.35 to 15.5 mg/kg with a mean of 11.1 (Table C-6).

**Prediction of Contaminant Phytoacculmation using Soil Property Data**
Statistical Regression Prediction Models
Multiple regression and Ridge regression models developed from the NCEA soil database for lettuce and NCEA plus SERDP soil databases for ryegrass tissue concentration are shown in Table C-7.  The PRESS statistic is reported for each ridge regression (RR) model.  Because PRESS is only used for RR and not the multiple regression models without ridge regression, the PRESS is 0 for MLR models in Table C-7.

For the models of toxins in lettuce, the PRESS statistic was significantly smaller in the ridge regression models than the MLR models indicating that reducing the influence of multicollinearity and allowing some bias in parameter estimates was beneficial for predictive ability.  For the models of toxins in grass, the ridge regression and MLR models produced similar PRESS statistics indicating that the ridge adjustment did not lead to much improvement in predictive ability.

Results of both MLR and RR equations are shown in Table C-7.  The MLR relationship for lettuce As vs. soil properties was not significant ($R^2 = 0.60$, P <0.247), while it was strongly significant for ryegrass As ($R^2 = 0.66$, P < 0.0001).   There was a weak relationship for lettuce Cd ($R^2 = 0.864$, P <0.32) while it was strongly significant for ryegrass Cd ($R^2 = 0.86$, P <0.001).  The relationship for lettuce Pb was weak ($R^2 = 0.73$, P <0.059) yet strong for ryegrass Pb ($R^2 =$

**Table C-6.** Pore water (1:1), calcium nitrate, and Mehlich 3 extractable

contaminants from NCEA and contaminated ESTCP soils.  Data was included
only if there is corresponding plant tissue data.

| Soil | Pore Water | | | Calcium Nitrate | | Mehlich 3 |
|------|------|------|------|------|------|------|
| | As | Cd | Pb | Cd | Pb | As |
| | ------------------------------------- mg/kg ------------------------------------- | | | | | |
| | NCEA | | | | | |
| CAN | 8.44 | 0.040 | 0.740 | 7.20 | 10.7 | 107 |
| DEN A | 0.300 | 1.44 | 4.48 | 36.4 | 598 | 16.1 |
| DEN B | 0.020 | . | 2.73 | 27.4 | 596 | 8.64 |
| DOUG | . | 4.70 | 31.93 | 36.8 | 800 | . |
| HAN | 13.9 | 0.450 | 1.32 | 15.2 | 70.3 | 107 |
| KIRK | 2.01 | 1.29 | 3.16 | 37.6 | 665 | 55.9 |
| LUT | 1.49 | 0.080 | 0.530 | 12.6 | 17.6 | 68.2 |
| MAN A | 16.9 | . | 0.750 | 6.50 | 26.1 | 145 |
| MANB | 20.3 | . | 0.150 | 5.00 | 40.5 | . |
| Norge | . | 9.19 | 56.07 | 32.9 | 990 | 57.4 |
| OSA | 0.230 | 0.160 | 1.70 | 19.9 | 69.1 | . |
| OSB | 0.120 | 0.270 | 0.640 | 23.3 | 74.4 | 17.4 |
| PCA | 7.49 | 1.82 | 13.21 | 33.1 | 5.11 | 73.1 |
| PCB | 0.49 | 0.430 | 7.21 | 43.8 | 705 | 59.6 |
| PRATA | . | . | 90.83 | 40.2 | 1085 | 97.5 |
| PRATB | . | 3.93 | 124.3 | 49.3 | 1047 | 114 |
| RICH | 8.65 | 0.200 | 4.44 | 19.6 | 151 | 164 |
| SUMA | 0.320 | 0.090 | 0.470 | 8.30 | 21.7 | 24.4 |
| SUMB | 0.020 | 0.030 | 0.170 | 20.8 | 78.3 | 23.3 |
| TALOKA | 3.81 | 4.86 | 21.47 | 42.4 | 726 | 59.2 |
| TELLER | 10.1 | 17.21 | 92.07 | 33.7 | 977 | 67.6 |
| min | 0.020 | 0.030 | 0.150 | 5.00 | 5.11 | 8.64 |
| max | 20.3 | 17.2 | 124 | 49.3 | 1085 | 164 |
| mean | 5.56 | 2.72 | 21.8 | 26.3 | 417 | 70.3 |
| | ESTCP | | | | | |
| Con | 0.199 | . | . | . | . | 12.8 |
| CP | . | 0.350 | . | 16.6 | . | . |
| Des | 1.19 | . | . | . | . | 7.35 |
| Hill | . | 0.033 | . | 3.84 | . | . |
| Hilo | 0.164 | <0.1 | 0.534 | <2 | 33.2 | 15.5 |
| MC | . | 0.869 | . | 15.5 | 26.7 | . |
| Mech | . | . | . | . | <2 | . |
| ORNL | . | . | 33.3 | . | 603 | . |
| Port | . | . | 5.53 | . | 507 | . |
| PC | <0.1 | . | . | . | <2 | 8.70 |

**Table C-6 (continued).** Pore water (1:1), calcium nitrate, and Mehlich 3

extractable contaminants from NCEA and contaminated ESTCP soils.  Data was
included only if there is corresponding plant tissue data.

| Soil | Pore Water | | | Calcium Nitrate | | Mehlich 3 |
|---|---|---|---|---|---|---|
| | As | Cd | Pb | Cd | Pb | As |
| | ------------------------------------ mg/kg ------------------------------------ | | | | | |
| Travis | . | . | 0.16 | . | 48.1 | . |
| min | <0.1 | <0.1 | 0.160 | <2 | <2 | 7.35 |
| max | 1.19 | 0.869 | 33.3 | 16.6 | 603 | 15.5 |
| mean | 0.518 | 0.417 | 9.88 | 12.0 | 244 | 11.1 |

**Table C-7.** Predictive equations to determine contaminant phytoacculumation from soil properties and total contaminant content. All equations have the form log plant tissue = intercept + a pH + b OC + c FEAL + d CEC + e Total. Values listed under pH, OC, FEAL, CEC, and Total are regression coefficients.

| | Model | Press | Intercept | pH | OC | FEAL | CEC | Total† | $R^2$ | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| Lettuce As | MLR | 0 | 6.51 | -0.278 | 0.471 | -2.49 | 0.0138 | -0.021 | 0.60 | <0.247 |
| | Ridge | 0.75 | 2.7 | -0.113 | 0.0924 | -0.244 | -0.0073 | 0.005 | | |
| Ryegrass As | MLR | 0 | 0.297 | 0.125 | 0.084 | -2.416 | -0.027 | 0.0031 | 0.66 | <0.0001 |
| | Ridge | 0.05 | 0.547 | 0.087 | 0.0293 | -2.95 | -0.184 | 0.0029 | | |
| Lettuce Cd | MLR | 0 | 2.44 | -0.119 | -0.145 | 0.432 | -0.0089 | 0.005 | 0.864 | <0.0318 |
| | Ridge | 0.65 | 2.42 | -0.0753 | -0.094 | -0.426 | -0.0065 | -0.0002 | | |
| Ryegrass Cd | MR | 0 | 1.55 | -0.043 | -0.167 | 0.241 | -0.0133 | 0.01189 | 0.856 | <0.0001 |
| | Ridge | 0.1 | 1.68 | -0.063 | -0.152 | -0.17 | -0.0104 | 0.0105 | | |
| Lettuce Pb | MLR | 0 | 1.79 | 0.0026 | -0.2 | -0.86 | -0.022 | 0.00022 | 0.725 | <0.0588 |
| | Ridge | 1.65 | 1.39 | -0.04 | -0.124 | -1.08 | -0.0077 | 0.00038 | | |
| Ryegrass Pb | MLR | 0 | 2.02 | -0.032 | -0.129 | -0.202 | -0.025 | 0.00033 | 0.817 | <0.0001 |
| | Ridge | 0.06 | 2.17 | -0.056 | -0.132 | -0.738 | -0.02 | 0.00031 | | |

† Total is the total contaminant soil content (mg/kg) for As, Cd, or Pb.

**Table C-7.** Summary of the Prediction of Contaminant Phytoaccumulation using Soil Property or Soil Extraction Soil Data

| Approach | Model or Soil Extraction | Ability to Predict Tissue As | | Ability to Predict Tissue Cd | | Ability to Predict Tissue Pb | |
|---|---|---|---|---|---|---|---|
| | | Lettuce | Ryegrass | Lettuce | Ryegrass | Lettuce | Ryegrass |
| Properties | MLR | 4 Concord Over , 5x | 4 Deseret Over, 80x | 4 Hill Under, 1.7x | 4 | 7 Portsmouth Over, 1.3x ORNL Under, 1.3x | 7 Portsmouth Under, 1.2x |
| | RR | 4 | 4 Deseret Over, 80x | 4 Hill Under, 1.7x | 4 | 7 Portsmouth Over, 2x ORNL Over, 2x | 7 Portsmouth Over, 1.7x |
| | | | | | | | |
| Soil Extraction | Pore water | 3 | 3 All sites Over, 2x | 3 | 3 Hill Under, 1.6x | 4 Portsmouth Under, 4x | 4 Portsmouth Under, 3.3x |
| | Mehlich 3 | 4 | 4 all sites Over, 2x to 5x | NA | NA | NA | NA |
| | Calcium Nitrate | NA | NA | 3 Hill Under, 10x | 3 Hill Under, 4x | 4 Portsmouth Under, 2x | 4 Portsmouth Under, 2.5x |

0.82, P <0.001).  In part, the stronger predictive equations for ryegrass vs. lettuce As, Cd, and Pb are due to differences in the size of their respective databases.  Lettuce multiple linear regression models were developed using only the NCEA soil properties whereas ryegrass models were developed from NCEA and SERDP spiked soils.  Therefore, there are more data for ryegrass than lettuce.  This resulted in generally weaker lettuce regression relationships than  ryegrass.  For lettuce As n = 12, while for ryegrass As n = 32.  For lettuce Cd n = 11, while for ryegrass Cd n = 26.  Finally for lettuce Pb n = 13, while for ryegrass Pb n = 37.

These models were developed to determine if they could predict ESTCP tissue contaminant content from ESTCP soil properties and contaminant soil content (i.e., total in Table C-7).  To illustrate the predictive ability of the developed models ESTCP tissue content was plotted against a 1:1 line generated from ESTCP tissue data.  The RR model closely predicted the tissue As for lettuce grown on ESTCP soils (Fig. C-1A).  With the exception of the Concord soil, the MLR model closely predicted the tissue As for lettuce grown on ESTCP soils (Fig. C-1A).   Both RR and MLR model closely predicted ryegrass tissue As with the exception of the Deseret soil (Fig C-1B.  The prediction models grossly over predicted tissue As content for Deseret.

Both RR and MLR models were able to predict lettuce Cd grown on ESTCP soils except for the Hill soil (Fig. C-2A).  The models under predicted tissue Cd  by approximately 1.7x for lettuce grown on the Hill soil.  Both RR and MLR models were able to predict ryegrass Cd content grown on ESTCP for all soils (Fig. C-2B).  For both lettuce and ryegrass Cd, ridge predictions were generally slightly better than MLR predictions.

Both RR and MLR models were able to predict lettuce Pb grown on ESTCP soils except for the Portsmouth and ORNL soils (Fig. C-3A).  The MLR under predicted lettuce tissue Pb about 1.3x and RR over predicted tissue Pb by 2x for the ORNL soil.  Both RR and MLR over predicted lettuce tissue Pb for the Portsmouth soil by about 2x and 1.3x, respectively.   Both RR and MLR models were able to predict ryegrass Pb content grown on ESTCP for all soils (Fig. C-3B).  However, RR over predicted tissue Pb about 1.7x while MLR slightly under predicted ryegrass tissue Pb 1.2x for the Portsmouth soil.

**Prediction of Contaminant Phytoacculumation using Soil Extraction Data**
Simple regression models, using the NCEA soil extraction and lettuce tissue data (Figs. C-4 to C-9) were developed to determine if soil extraction methods could be predictive of contaminant bioavailability.  Pore water extractable As was significantly related between lettuce tissue As ($r^2$ = 0.837, P < 0.01) (Fig. C-4A) and ryegrass tissue As ($r^2$ = 0.474, P < 0.01) (Fig. C-4B).  Soil test Mehlich 3 extractable As was a significantly related between lettuce tissue As ($r^2$ = 0.515, P < 0.05) (Fig. C-5A) and ryegrass tissue ($r^2$ = 0.416, P <0.05) (Fig. C-5B).

Pore water extractable Cd was significantly related between lettuce tissue Cd ($r^2$ = 0.658, P < 0.01) (Fig. C-6A) and ryegrass tissue Cd ($r^2$ = 0.678, P < 0.01) uptake (Fig. C-6B).  Similarly, calcium nitrate extractable Cd was significantly related between lettuce tissue Cd ($r^2$ = 0.411, P < 0.05) (Fig. C-7A) and ryegrass tissue Cd ($r^2$ = 0.459, P < 0.01) (Fig. C-7B).

Pore water extractable Pb there was significantly related between lettuce tissue Pb ($r^2 = 0.693$, P < 0.01) (Fig. C-8A) and ryegrass tissue Pb ($r^2 = 0.454$, P < 0.05) (Fig. C-8B). Similarly, calcium nitrate extractable Pb was significantly related between lettuce tissue Pb ($r^2 = 0.538$, P < 0.01) (Fig. C-9A) and ryegrass tissue Pb ($r^2 = 0.622$, P < 0.01) (Fig. C-9B).

The ESTCP soil extractable and plant tissue data were plotted on the simple regressions (Figs. C-4 to C-9) developed using the NCEA data to illustrate how well those models could predict contaminant uptake in plants grown on ESTCP soils. Pore water extractable As accurately predicted lettuce tissue As (Fig. P4A), but over estimated ryegrass tissue As by 2x (Fig. C-4B). Mehlich 3 extractable As slight under estimated ESTCP lettuce tissue As (Fig. C-5A) and over estimated ESTCP ryegrass As 2x to 5x (Fig. C-5B).

Pore water extractable Cd was able to predict ESTCP lettuce Cd (Fig. C-6A), but underestimated lettuce tissue uptake for the Hill soil by 1.6x. Pore water extractable Cd was a fairly good predictor of ryegrass tissue Cd (Fig. C-6B). Calcium nitrate extractable Cd was an accurate predictor of ESTCP lettuce tissue Cd except for the Hill site which was greatly underestimated (ca. 10x, Fig. C-7A). Calcium nitrate extractable Cd was an accurate predictor of ESTCP lettuce tissue Cd except for the Hill site (Fig. C-7B) which was underestimated by 4x.

Pore water extractable Pb was able to predict ESTCP lettuce Pb (Fig. C-8A),but underestimated tissue Pb for the Port soil by a factor of 4x. Similarly, pore water extractable Pb was able to predict ESTCP ryegrass Pb (Fig. C-8B), but underestimated tissue Pb for the Port soil by a factor of 3.3x. Calcium nitrate extractable Pb was able to predict ESTCP lettuce tissue Pb, except for the Port soil which was underestimated by a factor of 2x (Fig. C-9A). Similarly, calcium nitrate extractable Pb was able to predict ESTCP ryegrass tissue Pb, except for the Port soil which was underestimated by a factor of 2.5x (Fig. C-9B).

**Summary of the Predictive Capability of Soil Property and Soil Extraction Models**
The predictive capability required by a soil property / soil extraction models depends on the degree of accuracy of contaminant phytoaccumulation determined by the risk assessor. With some exceptions, both soil property and soil extraction models were able to predict phytoavailability at < 35% of the measured contaminant tissue value. In general, soil property models were predictive of tissue As, Cd, and Pb (Table C-7). Exception were Deseret for As (ryegrass), Hill for Cd (lettuce), and Portsmouth for Pb. Similar findings were found for soil extraction models. In general, soil extraction models were predictive of tissue As, Cd, and Pb. Exceptions were the same as found for soil property models but soil extractions over predicted ryegrass tissue As for all soils.

The predictive capability by soil property / soil extraction models by soil is summarized in Table C-8. Comparison of MLR with RR shows the predictive capability was similar. Predictive capability was improved by RR vs. MLR for Concord (As) and ORNL (Pb). In general predictive capability of soil extraction methods was adequate to excellent with the exception of Hill for Cd (lettuce) and Portsmouth for Pb. Similar predictive capabilities were found for pore water vs. calcium nitrate extraction for Pb and Cd (Table C-8).
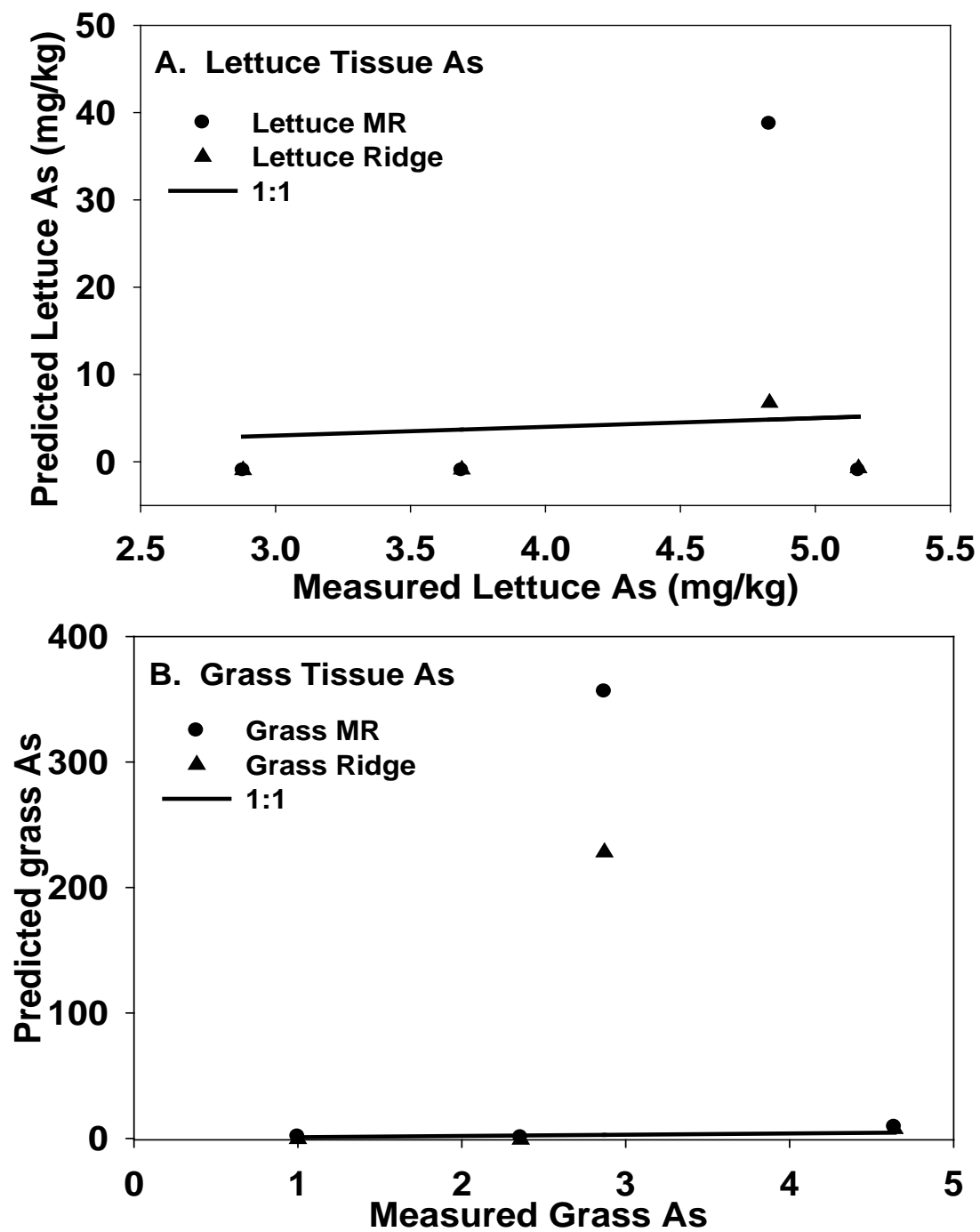
**Figure C-1.** Comparison of Predicted Lettuce (A) and Ryegrass (B) Arsenic Concentration from Multiple Regression and Ridge Regression with Measured Plant Concentrations
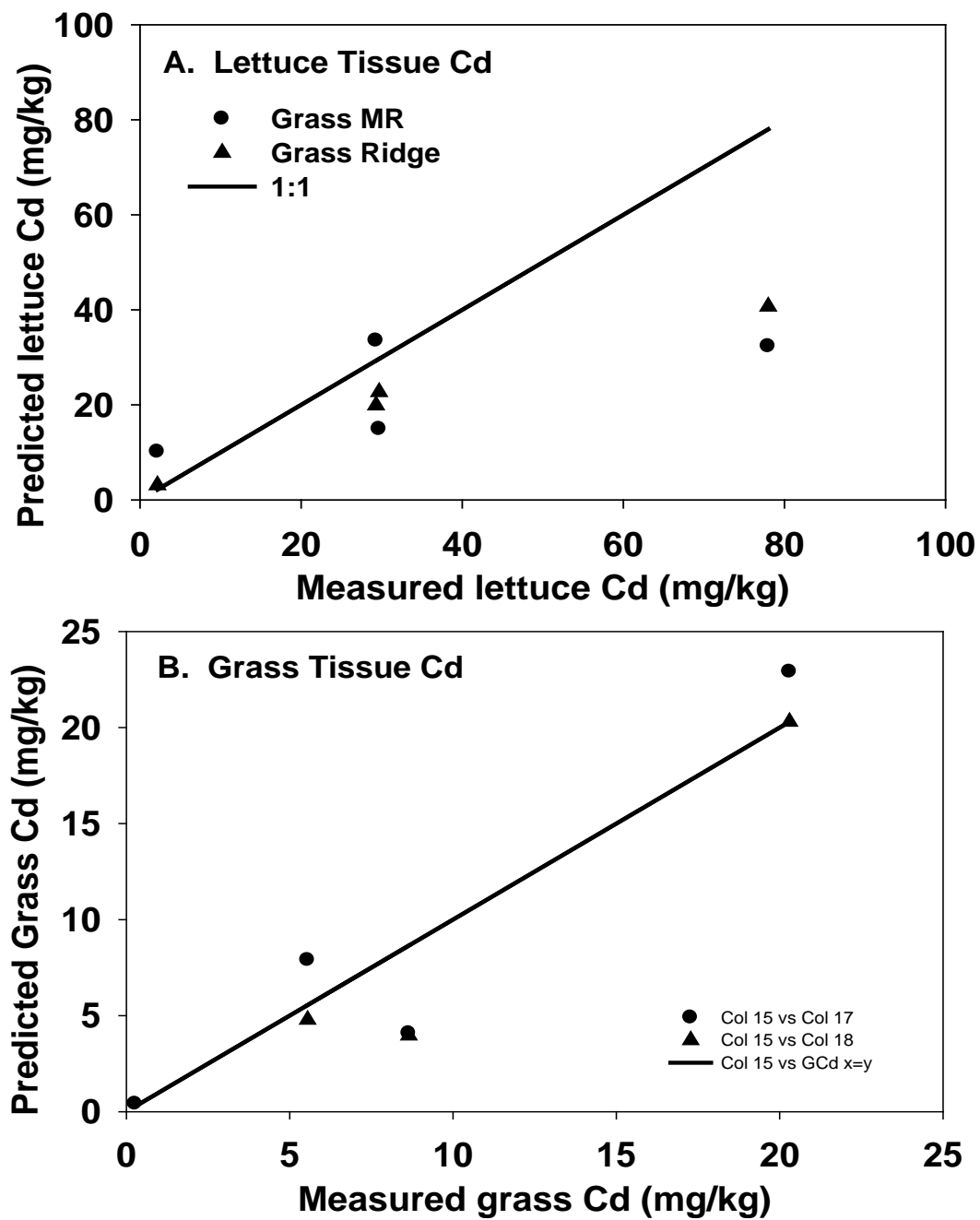
**Figure C-2.** Comparison of Predicted Lettuce (A) and Ryegrass (B) Cadmium Concentration from Multiple Regression and Ridge Regression with Measured Plant Concentrations
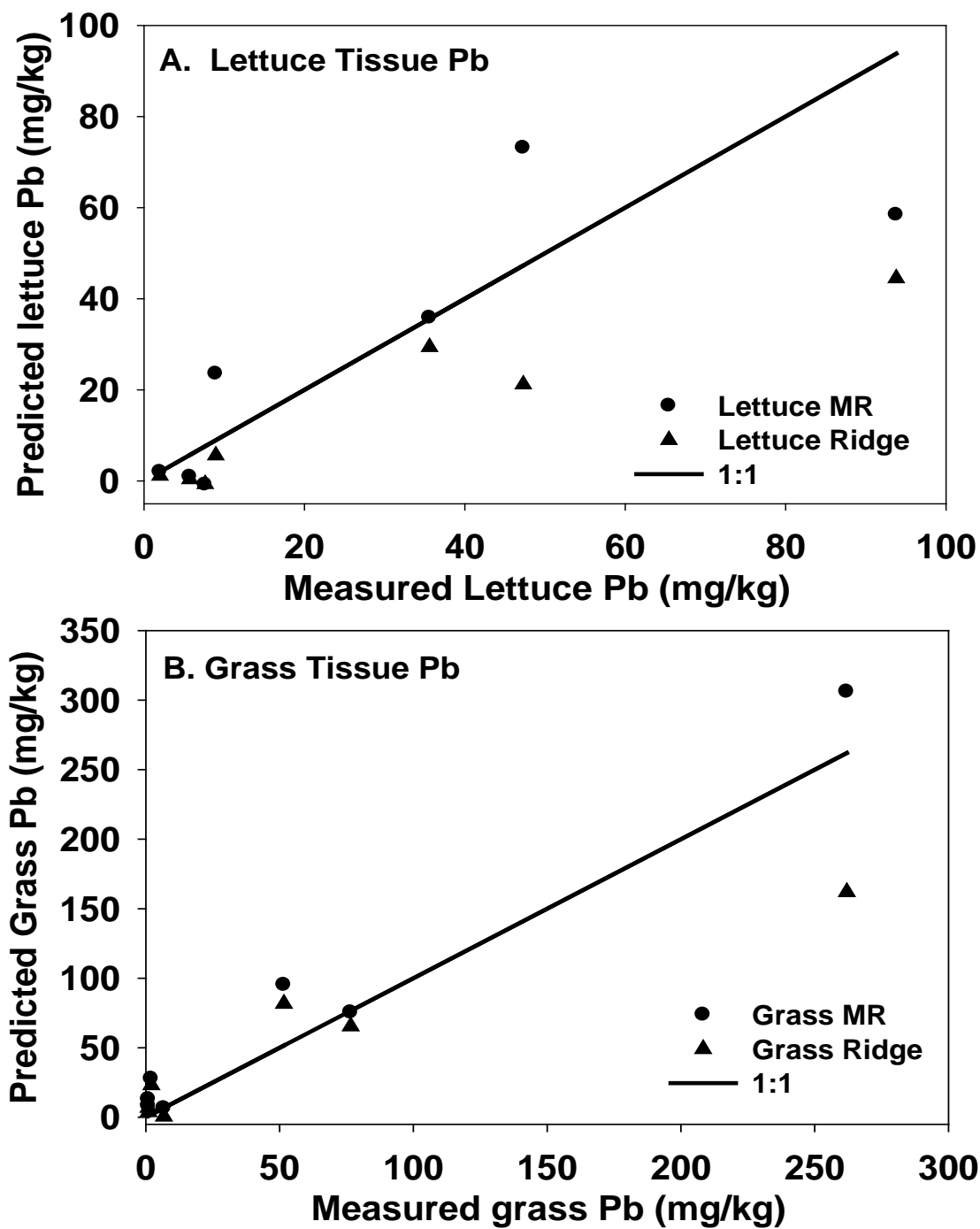
**Figure C-3.** Comparison of Predicted Lettuce (A) and Ryegrass (B) Lead Concentration from Multiple Regression and Ridge Regression with Measured Plant Concentrations
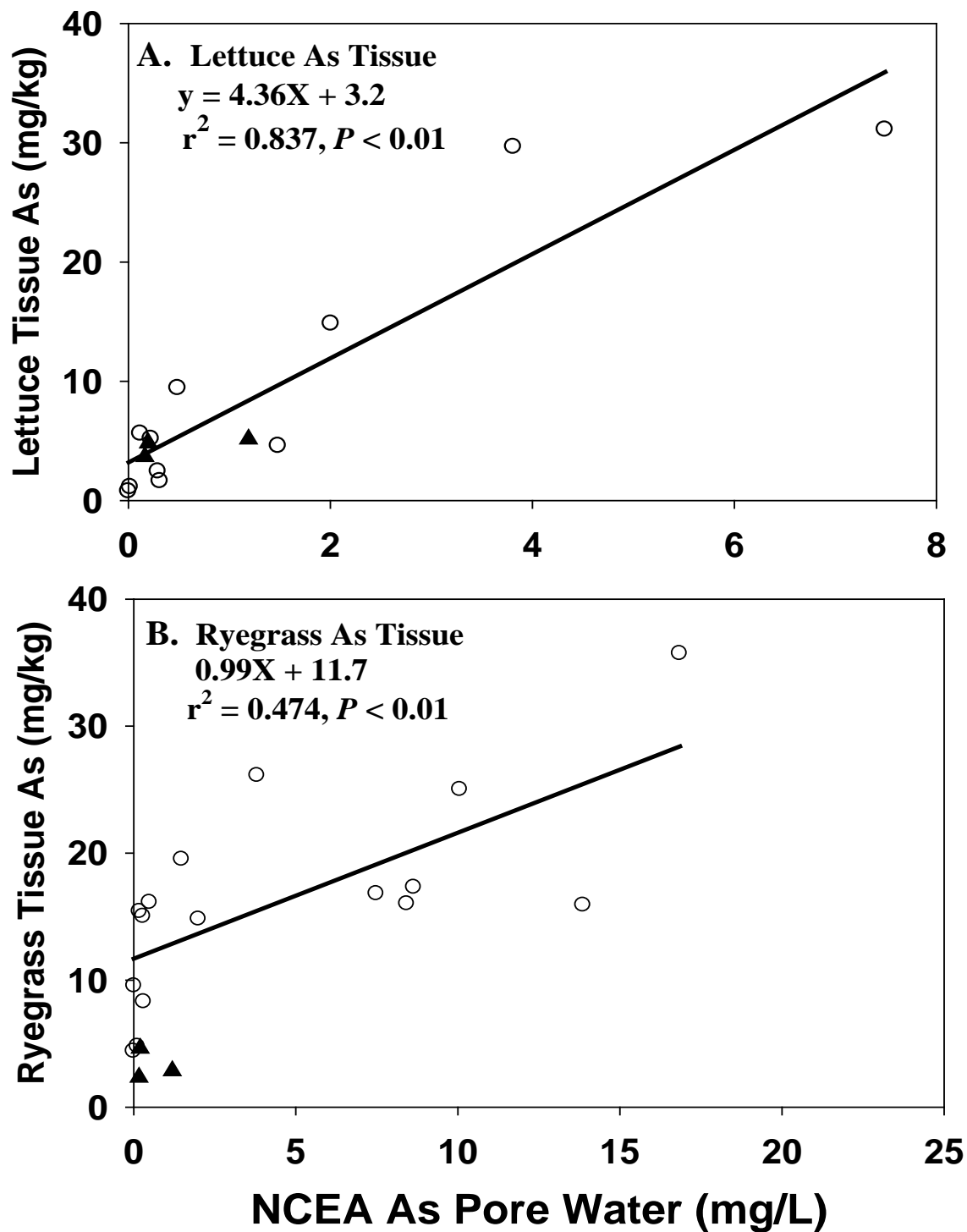
**Figure C-4.** Relationship between Lettuce (A) and Ryegrass (B) As concentration and Soil Pore Water As for NCEA soils.
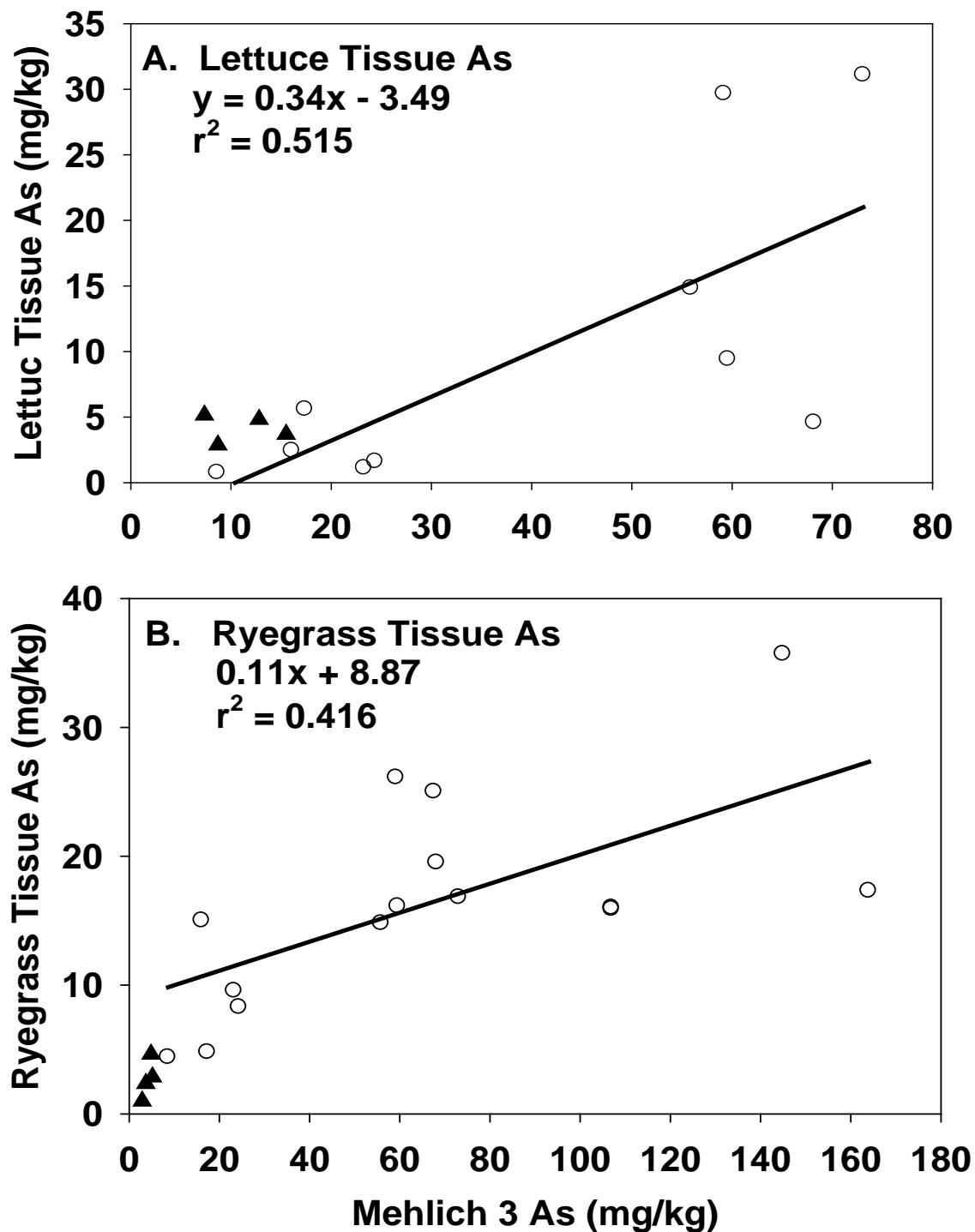
**Figure C-5.** Relationship between Lettuce (A) and Ryegrass (B) As concentration and Soil Mehlich 3 Extractable As for NCEA soils.
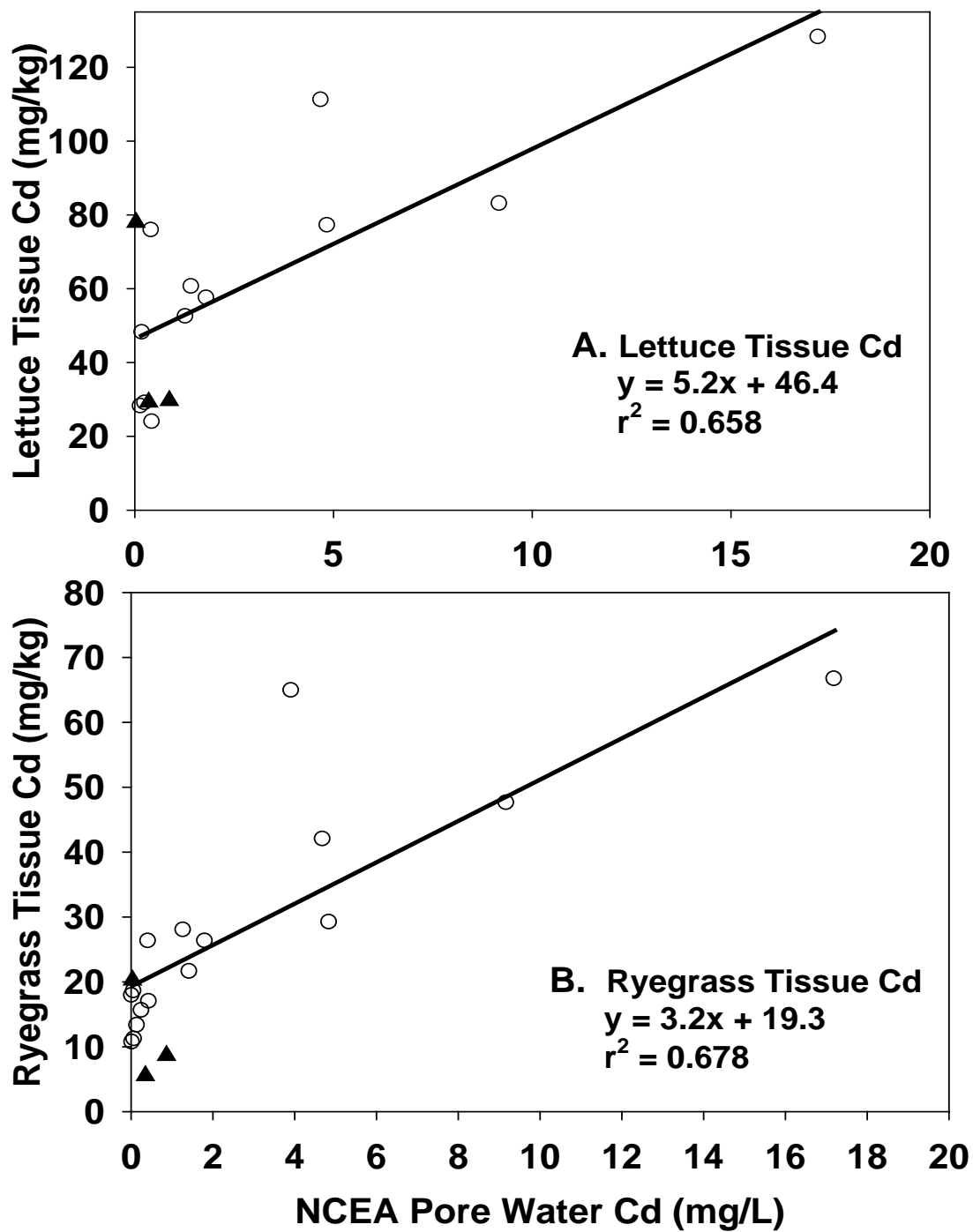
**Figure C-6.** Relationship between Lettuce (A) and Ryegrass (B) As concentration and Soil Pore Water Cd for NCEA soils.
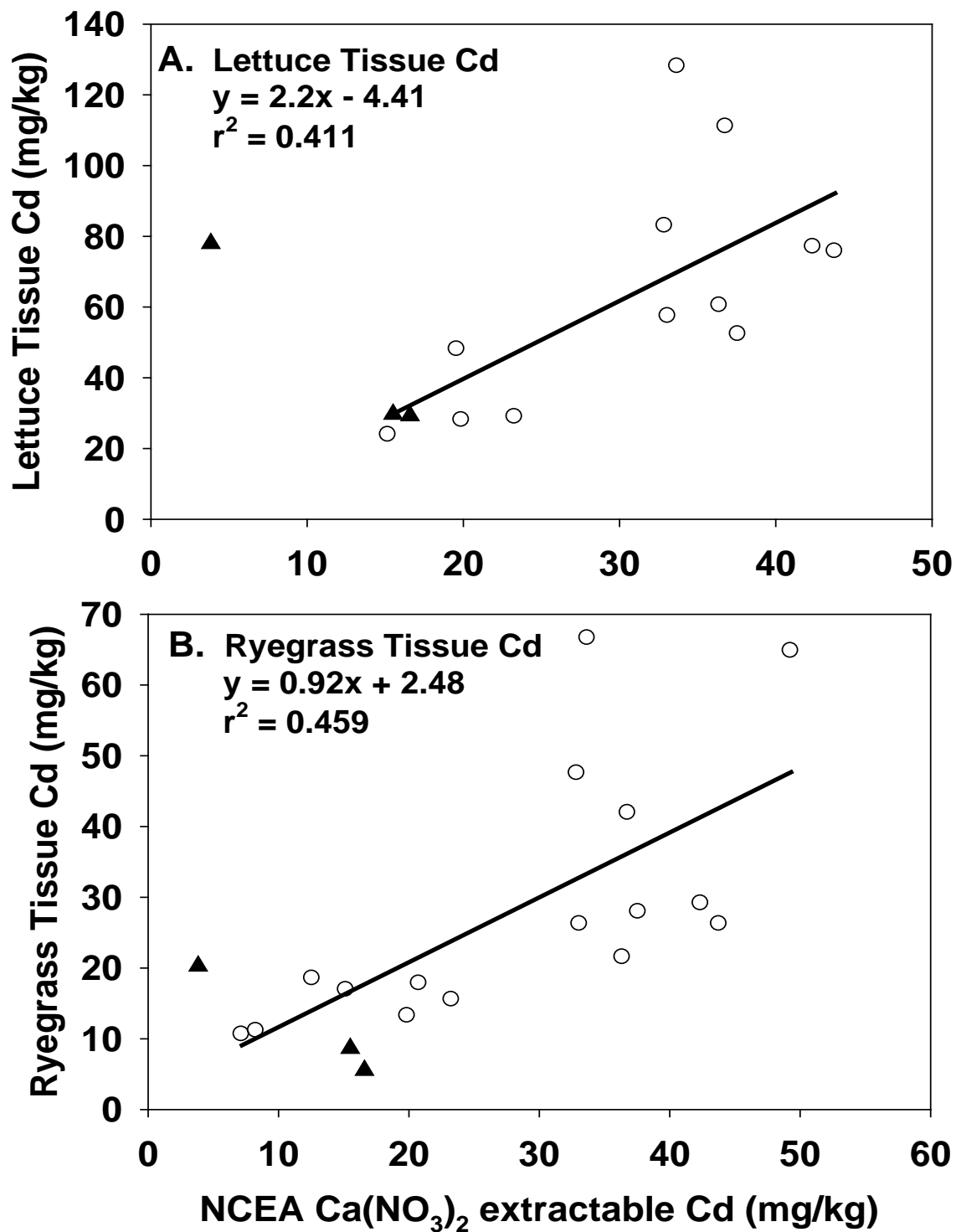
**Figure C-7.** Relationship between Lettuce (A) and Ryegrass (B) As Concentration and Soil 0.1 M Calcium Nitrate Extractable Cd for NCEA soils.
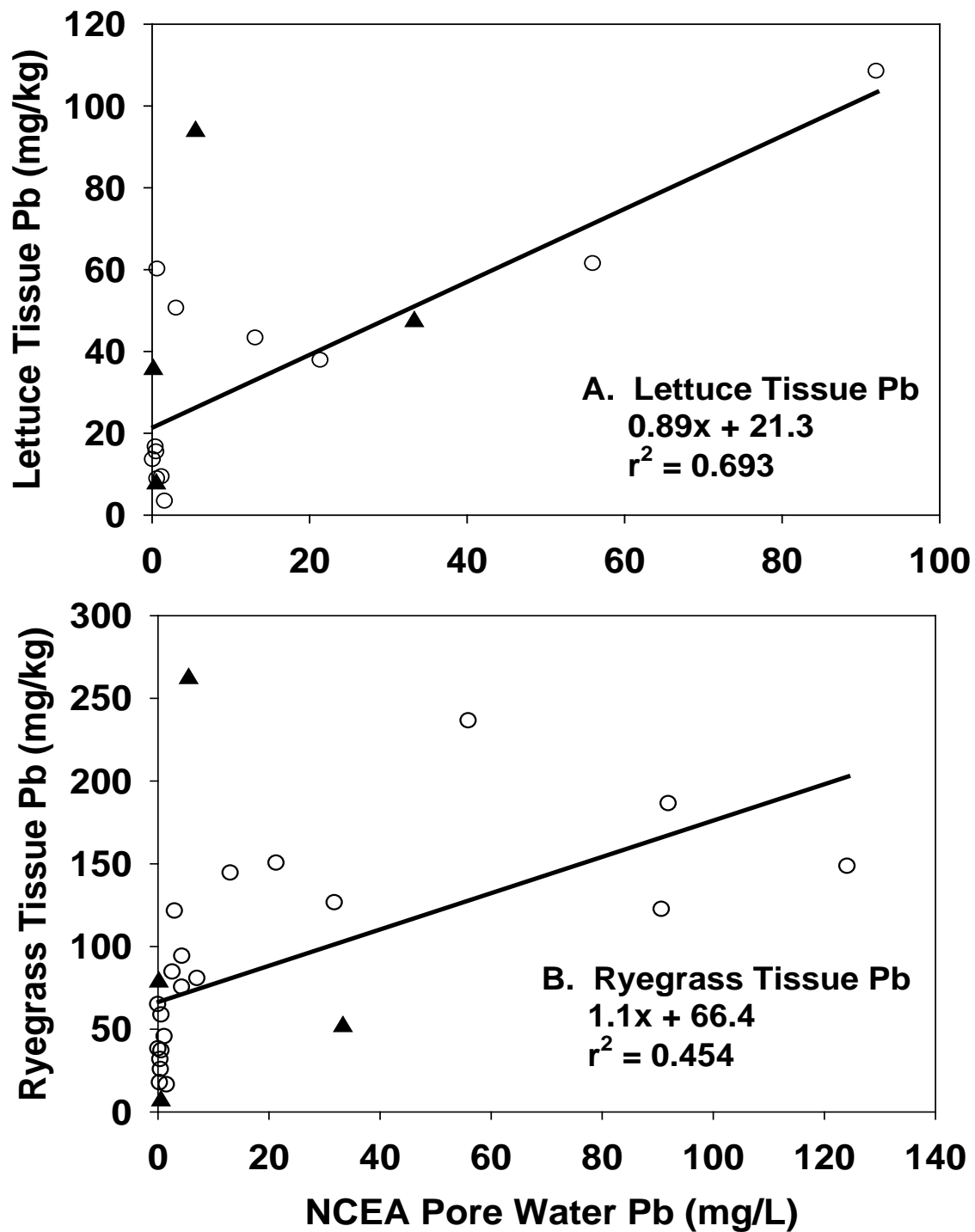
**Figure C-8.** Relationship between Lettuce (A) and Ryegrass (B) As concentration and Soil Pore Water Pb for NCEA soils.
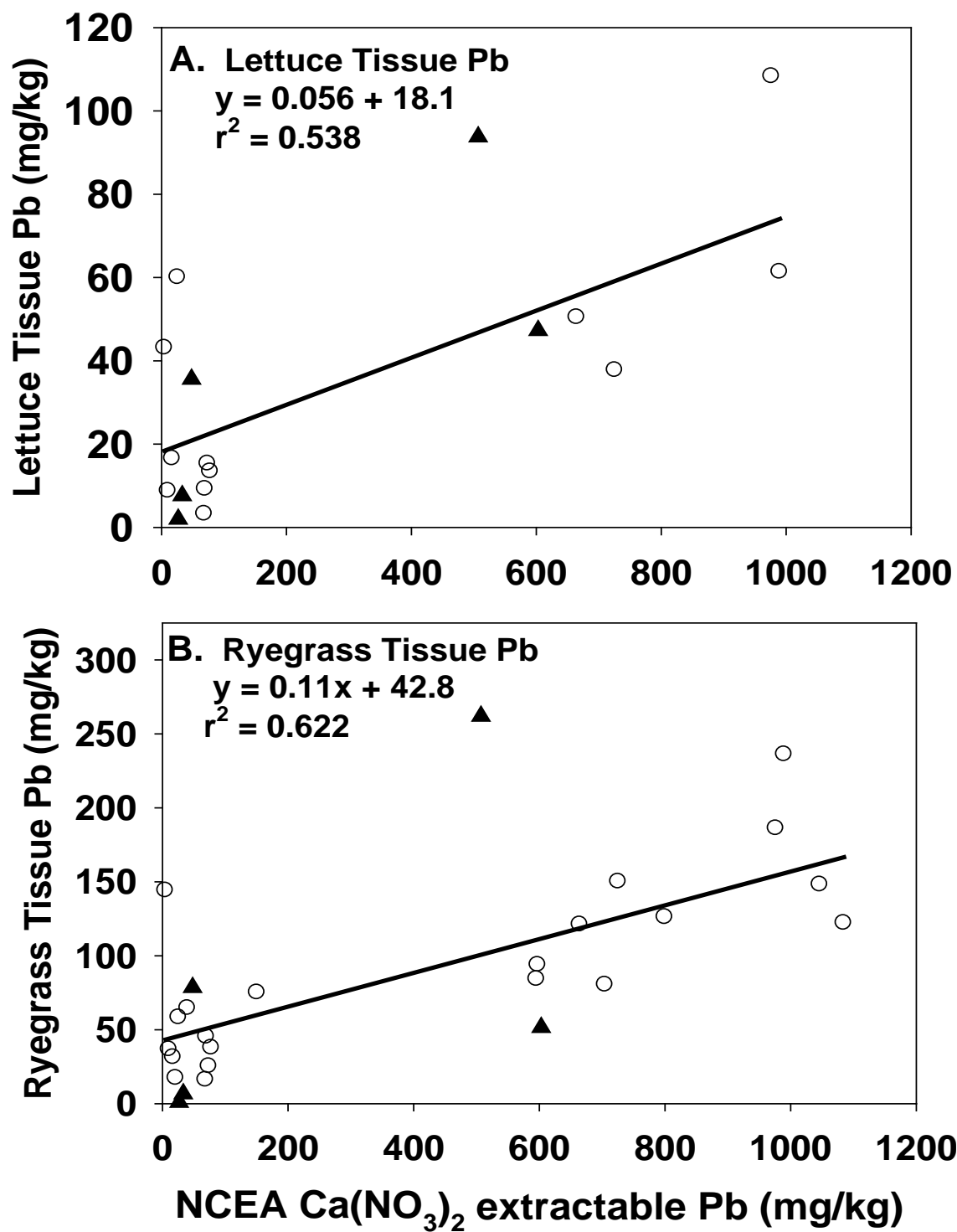
**Figure C-9.** Relationship between Lettuce (A) and Ryegrass (B) As Concentration and Soil 0.1 M Calcium Nitrate Extractable Pb for NCEA soils.

Table C-8. **Summary of the Prediction Capability†of Contaminant Phytoacculumation using Soil Property or Soil Extraction Soil Data, by soil**

| Soil | | Soil Property Model | | Soil Extraction Model | | |
|---|---|---|---|---|---|---|
| | | MLR | RR | Pore Water | Mehlich 3 | Ca(NO3)2 |
| Con | As | P | E | E (lettuce) A (rye) | E (lettuce) A (rye) | |
| | | | | | | |
| CP | As | A | A | | | |
| | Cd | | | A | | A |
| | | | | | | |
| Des | As | P | P | E (lettuce) A (rye) | E (lettuce) A (rye) | |
| | | | | | | |
| Hill | Cd | P | A | P (lettuce) E (rye) | | P (lettuce) A (rye) |
| | | | | | | |
| Hilo | As | A | A | E (lettuce) A (rye) | E (lettuce) A (rye) | |
| | Pb | A | A | | | |
| | | | | | | |
| MC | Cd | E | E | A | | A |
| | Pb | E | E | | | A |
| | | | | | | |
| Mech | Pb | E | E | | | |
| | | | | | | |
| ORNL | Pb | P (lettuce) ‡ A (rye) | E | A | | A |
| | | | | | | |
| PC | As | E | E | | E (lettuce) A (rye) | |
| | Pb | E | E | | | |
| | | | | | | |
| Port | Pb | P (lettuce) A (rye) | P (lettuce) A (rye) | P | | P |
| | | | | | | |
| Travis | Pb | E | E | A | | A |
| | | | | | | |

† Predictive capability, E = excellent, A=adequate, P=poor.
‡ Poor for lettuce, adequate for ryegrass

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## APPENDIX D

## RELATIVE BIOAVAILABILITY OF AS, PB, AND /OR CR IN SOIL

Relative bioavailability of Pb or As or Cr were determined in 3 study soils from juvenile swine dosing trials. Relative bioavailable Pb was determined for soil collected from the Portsmouth Naval Shipyard. Relative bioavailable As was determined for soil collected from the Deseret Chemical Depot. Relative bioavailable Cr was determined for soil collected from the McClellan Air Force Base.

### Relative bioavailable Pb of soil from the Portsmouth Naval Shipyard

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of lead from a sample collected from the Portsmouth Naval Shipyard. The test material contained a lead concentration of 4113 μg/g. The relative bioavailability of lead in the sample was assessed by comparing the absorption of lead from the test material to that of a reference material (lead acetate). Groups of five swine were given oral doses of lead acetate or test material twice a day for 14 days. The amount of lead absorbed by each animal was evaluated by measuring the amount of lead in the blood (measured on days 0, 3, 7, 9, 12, and 15) and the amount of lead in bone (measured on day 15 at study termination). The amount of lead present in blood or bone of animals exposed to test material was compared to that for animals exposed to lead acetate, and the results were expressed as relative bioavailability (RBA). The RBA results for the sample in this study are summarized in Table D-1. The lead RBA estimates are approximately 99% for the test material. This value is higher than the default value for lead in soil that is usually employed when reliable site-specific data are lacking. This indicates that the lead in this material is about as well absorbed as soluble lead. This relative bioavailability estimate may be used to improve accuracy and decrease uncertainty in estimating human health risks from exposure to this site-specific test material. Supporting detailed information of experimental data used to derive the RBA Pb value for the Portsmouth soil follows Table D-1.

### Relative bioavailable As of soil the Deseret Chemical Depot

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from a soil sample taken in the vicinity of the Deseret Chemical Depot The soil sample contained an arsenic concentration of 521 ug/g. The relative bioavailability of arsenic was assessed by comparing the absorption of arsenic from the test material to that of a reference material (sodium arsenate). Groups of five swine were given oral doses of sodium arsenate or the test materials twice a day for 14 days; a group of three non-treated swine served as a control. The amount of arsenic absorbed by each animal was evaluated by measuring the amount of arsenic excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The urinary excretion fraction (UEF) (the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours) was calculated for both the test soil and sodium arsenate using linear regression analysis.

**Table D-1.** Relative Bioavailable Pb of the Portsmouth Soil

### NAVY3 Lead RBA Estimates (All Data)

| Measurement Endpoint | Estimated Soil RBA (90% Confidence Interval) |
|---|---|
| Blood Lead AUC | 1.02 (0.63 - 1.37) |
| Femur Lead | 0.95 (0.71 - 1.30) |
| Point Estimate | 0.99 (0.70 - 1.27) |

## TABLE D-2 IDENTIFICATION OF POTENTIAL BLOOD LEAD OUTLIERS

| Material Administered | Group | Pig Number | Target Dose | Blood Lead (µg/dL) by Day | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 | 3 | 7 | 9 | 12 | 15 |
| Lead Acetate | 1 | 161 | 25 | 0.5 | 0.5 | 0.5 | 1.0 | 2.0 | 0.5 |
| Lead Acetate | 1 | 168 | 25 | 0.5 | 0.5 | 0.5 | 1.0 | 2.0 | 1.0 |
| Lead Acetate | 1 | 174 | 25 | 0.5 | 1.0 | 0.5 | 2.0 | 2.0 | 0.5 |
| Lead Acetate | 1 | 254 | 25 | 1.0 | 2.0 | 2.0 | 2.0 | 3.7 | 0.5 |
| Lead Acetate | 1 | 266 | 25 | 0.5 | 1.0 | 0.5 | 1.0 | 2.0 | 0.5 |
| Lead Acetate | 2 | 164 | 75 | 0.5 | 3.0 | 3.7 | 3.3 | 5.6 | 4.5 |
| Lead Acetate | 2 | 167 | 75 | 0.5 | 1.0 | 2.0 | 3.0 | 4.0 | 2.0 |
| Lead Acetate | 2 | 257 | 75 | 0.5 | 1.0 | 1.0 | 1.0 | 4.3 | 3.0 |
| Lead Acetate | 2 | 260 | 75 | 0.5 | 2.0 | 4.1 | 3.9 | 4.8 | 3.8 |
| Lead Acetate | 2 | 273 | 75 | 1.0 | 3.7 | 4.8 | 4.2 | 6.3 | 5.7 |
| Lead Acetate | 3 | 169 | 225 | 0.5 | 4.4 | 6.5 | 4.3 | 5.6 | 7.5 |
| Lead Acetate | 3 | 173 | 225 | 0.5 | 3.5 | 6.9 | 5.6 | 7.6 | 7.9 |
| Lead Acetate | 3 | 256 | 225 | 0.5 | 4.2 | 5.0 | 7.0 | 6.9 | 7.1 |
| Lead Acetate | 3 | 262 | 225 | 0.5 | 3.1 | 5.3 | 5.3 | 6.6 | 6.4 |
| Lead Acetate | 3 | 270 | 225 | 0.5 | 3.1 | **12.0** | 4.0 | 5.3 | 5.4 |
| Test Material 3 | 4 | 162 | 75 | 0.5 | 2.0 | 2.0 | 2.0 | 3.3 | 3.0 |
| Test Material 3 | 4 | 251 | 75 | 0.5 | 3.2 | 3.2 | 3.0 | 3.4 | 3.0 |
| Test Material 3 | 4 | 252 | 75 | 0.5 | 1.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Test Material 3 | 4 | 261 | 75 | 0.5 | 1.0 | 3.0 | 3.5 | 3.4 | 3.4 |
| Test Material 3 | 4 | 264 | 75 | 0.5 | 1.0 | 2.0 | 3.0 | 3.1 | 3.5 |
| Test Material 3 | 5 | 163 | 225 | 1.0 | 3.9 | 6.6 | 6.1 | 7.3 | 4.9 |
| Test Material 3 | 5 | 171 | 225 | 0.5 | 4.9 | 6.8 | 5.3 | 6.5 | 6.7 |
| Test Material 3 | 5 | 255 | 225 | 0.5 | 3.6 | 4.7 | 6.2 | 7.1 | 6.5 |
| Test Material 3 | 5 | 263 | 225 | 0.5 | 3.7 | 7.7 | 7.1 | 8.1 | 7.8 |
| Test Material 3 | 5 | 267 | 225 | 0.5 | 5.7 | 9.2 | 9.0 | 9.2 | 8.6 |
| Test Material 3 | 6 | 170 | 675 | 0.5 | 7.9 | 12.0 | 11.0 | 12.0 | 13.0 |
| Test Material 3 | 6 | 258 | 675 | 0.5 | 11.0 | 14.0 | 14.0 | 14.0 | 17.0 |
| Test Material 3 | 6 | 265 | 675 | 0.5 | 7.8 | 12.0 | 11.0 | 9.6 | 12.0 |
| Test Material 3 | 6 | 268 | 675 | 0.5 | 11.0 | 12.0 | 11.0 | 14.0 | 12.0 |
| Test Material 3 | 6 | 269 | 675 | 0.5 | 7.5 | 7.0 | **0.5** | 7.0 | 9.6 |
| Control | 7 | 165 | 0 | 0.5 | 0.5 | 0.5 | 0.5 | **13.0** | 0.5 |
| Control | 7 | 172 | 0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Control | 7 | 271 | 0 | 0.5 | 3.4 | 0.5 | **7.9** | 0.5 | 0.5 |

Dose units: µg/kg-d

▢ Data point judged to be outlier; excluded from further analyses

# FIGURE D-1  BLOOD LEAD DATA BY DAY
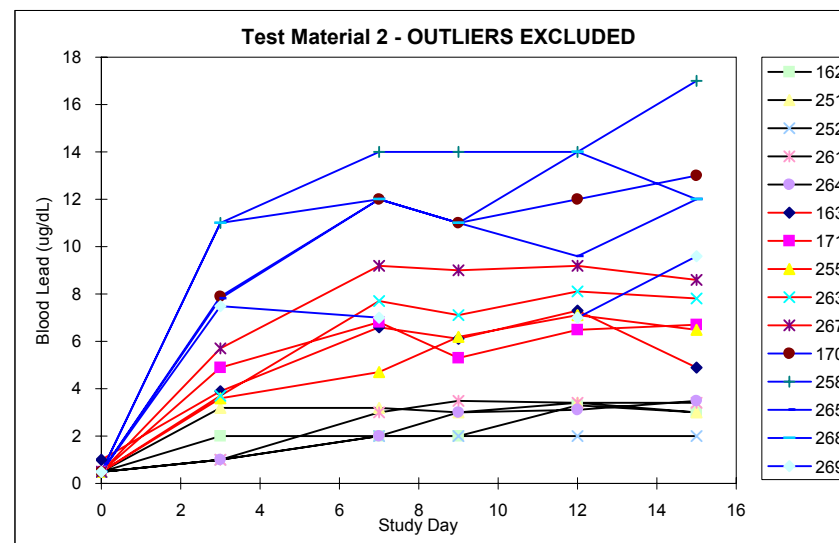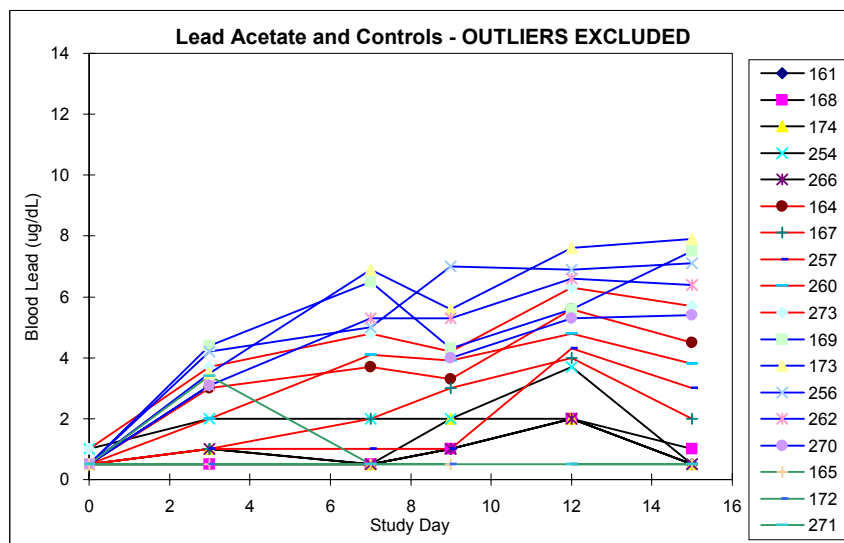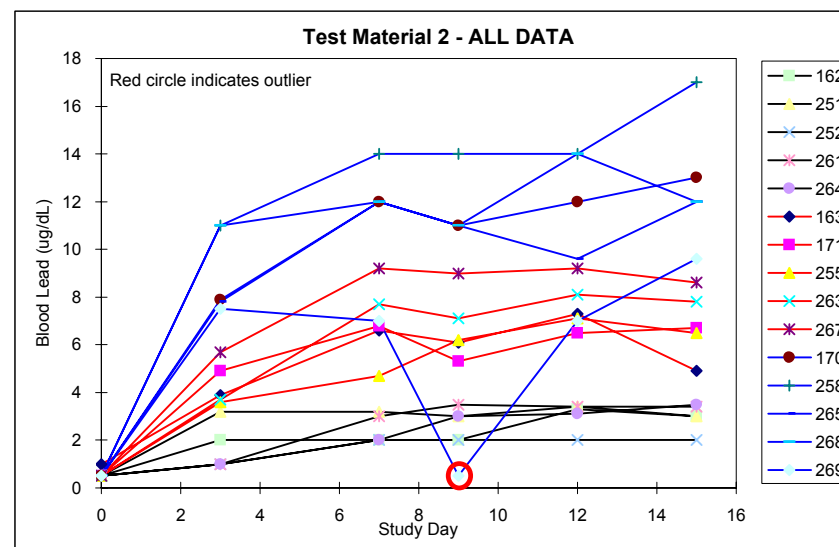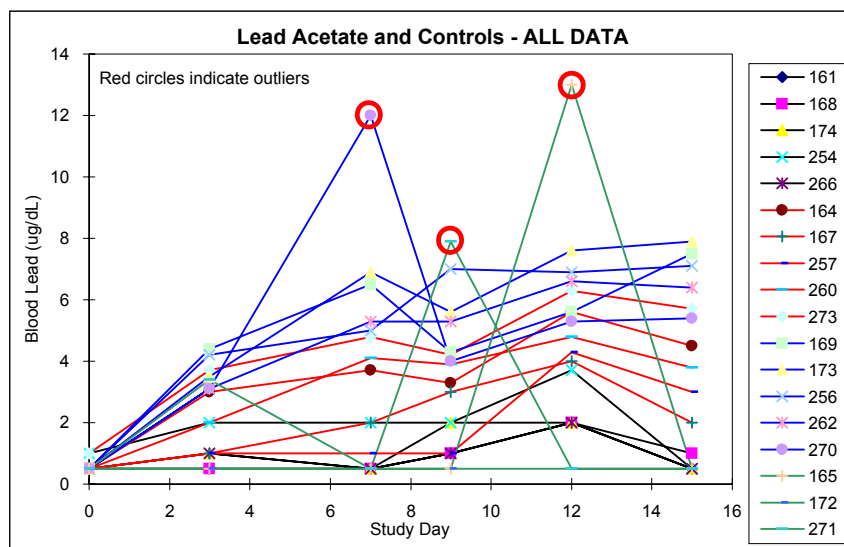
# FIGURE D-2  GROUP MEAN BLOOD LEAD BY DAY



**All Data**

Legend:
- 1 (Lead Acetate 25)
- 2 (Lead Acetate 75)
- 3 (Lead Acetate 225)
- 4 (Test Material 3 75)
- 5 (Test Material 3 225)
- 6 (Test Material 3 675)
- 7 (Control 0)

**Outliers Excluded**

Legend:
- 1 (Lead Acetate 25)
- 2 (Lead Acetate 75)
- 3 (Lead Acetate 225)
- 4 (Test Material 3 75)
- 5 (Test Material 3 225)
- 6 (Test Material 3 675)
- 7 (Control 0)
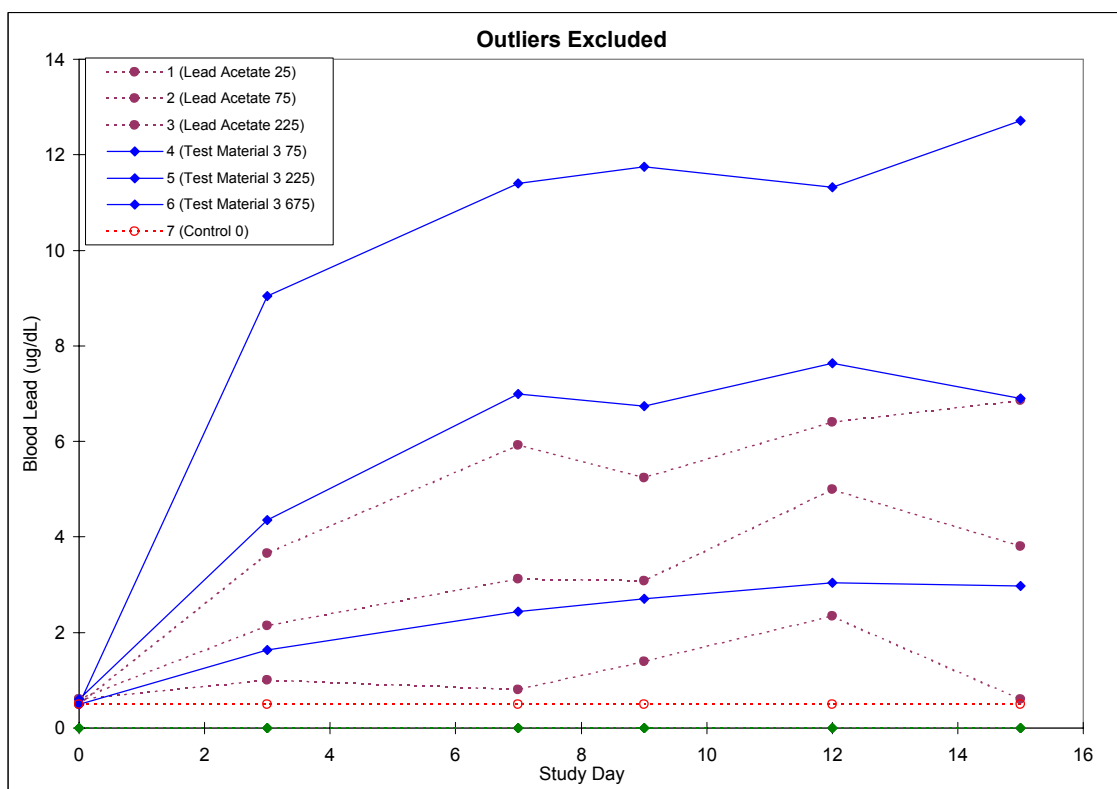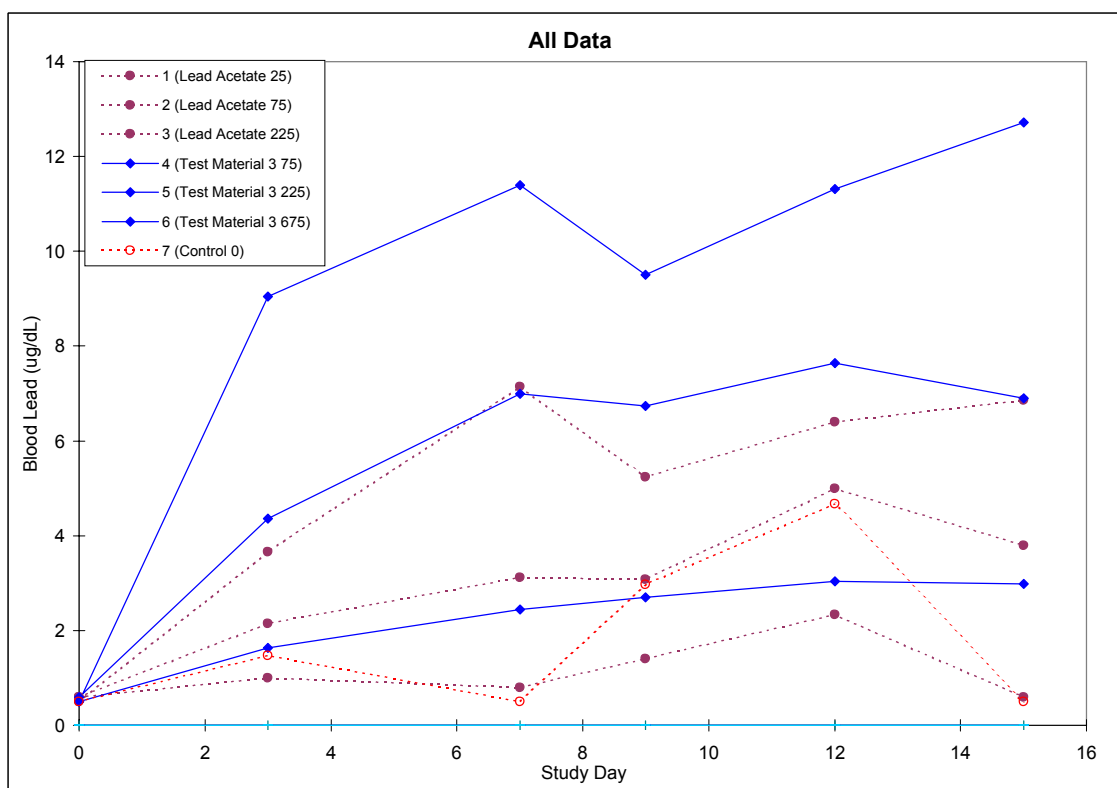
## TABLE D-3  BLOOD LEAD AREA UNDER CURVE DETERMINATIONS

| Group | Pig Number | AUC (µg/dL-days) for Time Interval Shown | | | | | AUC Total (µg/dL-days) |
|---|---|---|---|---|---|---|---|
| | | 0-3 | 3-7 | 7-9 | 9-12 | 12-15 | |
| 1 | 161 | 1.50 | 2.00 | 1.50 | 4.50 | 3.75 | 13.25 |
| 1 | 168 | 1.50 | 2.00 | 1.50 | 4.50 | 4.50 | 14.00 |
| 1 | 174 | 2.25 | 3.00 | 2.50 | 6.00 | 3.75 | 17.50 |
| 1 | 254 | 4.50 | 8.00 | 4.00 | 8.55 | 6.30 | 31.35 |
| 1 | 266 | 2.25 | 3.00 | 1.50 | 4.50 | 3.75 | 15.00 |
| 2 | 164 | 5.25 | 13.40 | 7.00 | 13.35 | 15.15 | 54.15 |
| 2 | 167 | 2.25 | 6.00 | 5.00 | 10.50 | 9.00 | 32.75 |
| 2 | 257 | 2.25 | 4.00 | 2.00 | 7.95 | 10.95 | 27.15 |
| 2 | 260 | 3.75 | 12.20 | 8.00 | 13.05 | 12.90 | 49.90 |
| 2 | 273 | 7.05 | 17.00 | 9.00 | 15.75 | 18.00 | 66.80 |
| 3 | 169 | 7.35 | 21.80 | 10.80 | 14.85 | 19.65 | 74.45 |
| 3 | 173 | 6.00 | 20.80 | 12.50 | 19.80 | 23.25 | 82.35 |
| 3 | 256 | 7.05 | 18.40 | 12.00 | 20.85 | 21.00 | 79.30 |
| 3 | 262 | 5.40 | 16.80 | 10.60 | 17.85 | 19.50 | 70.15 |
| 3 | 270 | 5.40 | 13.60 | 7.70 | 13.95 | 16.05 | 56.70 |
| 4 | 162 | 3.75 | 8.00 | 4.00 | 7.95 | 9.45 | 33.15 |
| 4 | 251 | 5.55 | 12.80 | 6.20 | 9.60 | 9.60 | 43.75 |
| 4 | 252 | 2.25 | 6.00 | 4.00 | 6.00 | 6.00 | 24.25 |
| 4 | 261 | 2.25 | 8.00 | 6.50 | 10.35 | 10.20 | 37.30 |
| 4 | 264 | 2.25 | 6.00 | 5.00 | 9.15 | 9.90 | 32.30 |
| 5 | 163 | 7.35 | 21.00 | 12.70 | 20.10 | 18.30 | 79.45 |
| 5 | 171 | 8.10 | 23.40 | 12.10 | 17.70 | 19.80 | 81.10 |
| 5 | 255 | 6.15 | 16.60 | 10.90 | 19.95 | 20.40 | 74.00 |
| 5 | 263 | 6.30 | 22.80 | 14.80 | 22.80 | 23.85 | 90.55 |
| 5 | 267 | 9.30 | 29.80 | 18.20 | 27.30 | 26.70 | 111.30 |
| 6 | 170 | 12.60 | 39.80 | 23.00 | 34.50 | 37.50 | 147.40 |
| 6 | 258 | 17.25 | 50.00 | 28.00 | 42.00 | 46.50 | 183.75 |
| 6 | 265 | 12.45 | 39.60 | 23.00 | 30.90 | 32.40 | 138.35 |
| 6 | 268 | 17.25 | 46.00 | 23.00 | 37.50 | 39.00 | 162.75 |
| 6 | 269 | 12.00 | 29.00 | 14.00 | 21.00 | 24.90 | 100.90 |
| 7 | 165 | 1.50 | 2.00 | 1.00 | 1.50 | 1.50 | 7.50 |
| 7 | 172 | 1.50 | 2.00 | 1.00 | 1.50 | 1.50 | 7.50 |
| 7 | 271 | 5.85 | 7.80 | 1.00 | 1.50 | 1.50 | 17.65 |

# FIGURE D-3  VARIANCE MODELS



**BLOOD AUC**

Legend:
- ◆ Model - Lead Acetate
- △ Model - Test Materials
- ● Navy3 - Lead Acetate
- ● Navy3 - Test Material

y-axis: ln(Group Variance)
x-axis: ln(Group Mean Response)

Variance Model:
y = 1.5516x - 1.3226
R2 = 0.5046
p-value < 0.01

**FEMUR**

Legend:
- ◆ Model - Lead Acetate
- △ Model - Test Materials
- ● Navy3 - Lead Acetate
- ● Navy3 - Test Material

y-axis: ln(Group Variance)
x-axis: ln(Group Mean Response)

Variance Model:
y = 1.656x - 1.9713
R2 = 0.7022
p-value < 0.01

4_MSE3 Variance.xls (Fig4-2_All Endpoints)

# FIGURE D-4  SAMPLE PREPARATION REPLICATES



**Panel A - Blood Lead**

**Panel B - Femur Lead**
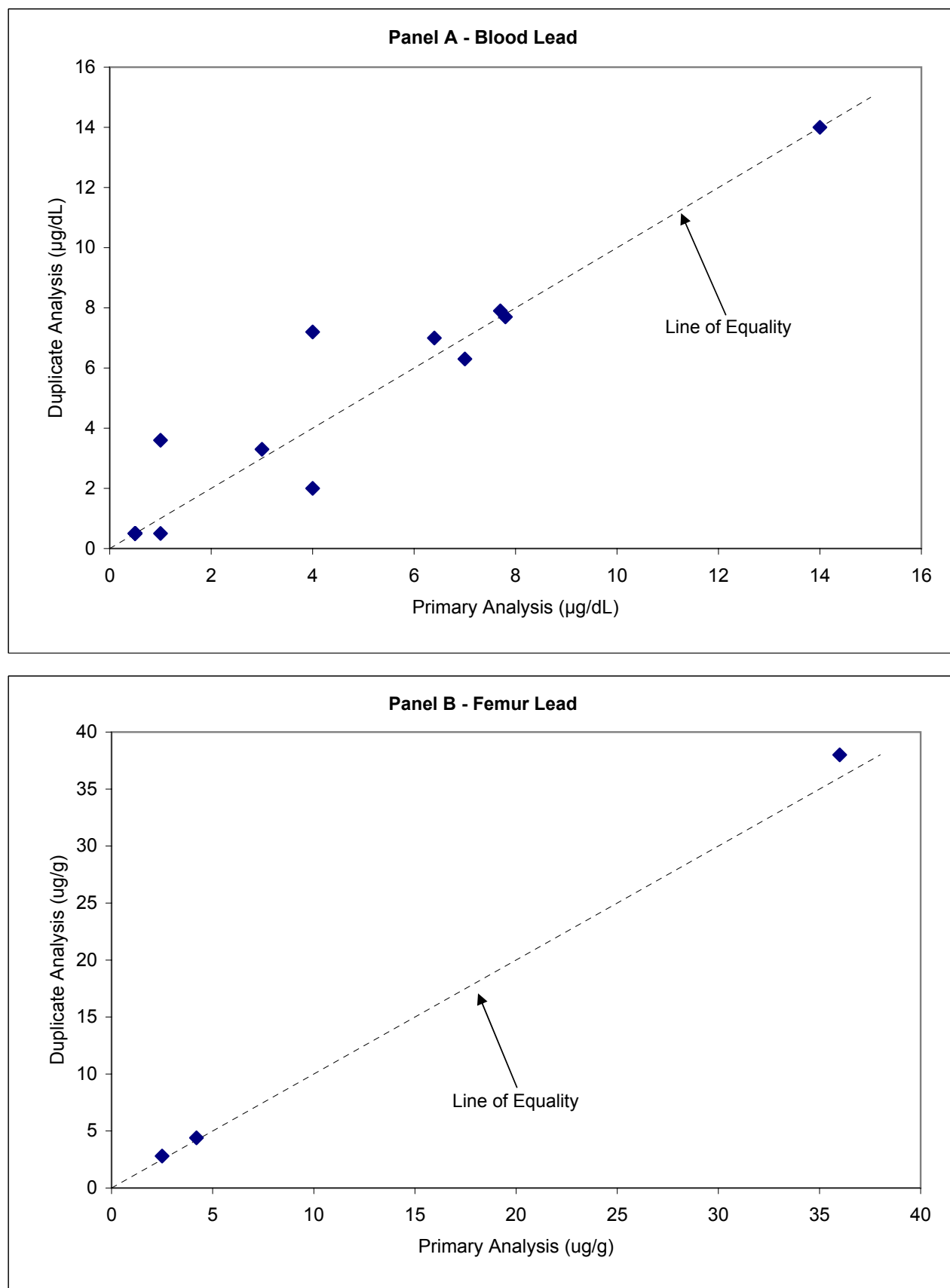
**FIGURE D-5  CDC BLOOD LEAD CHECK SAMPLES**

Line of Equality
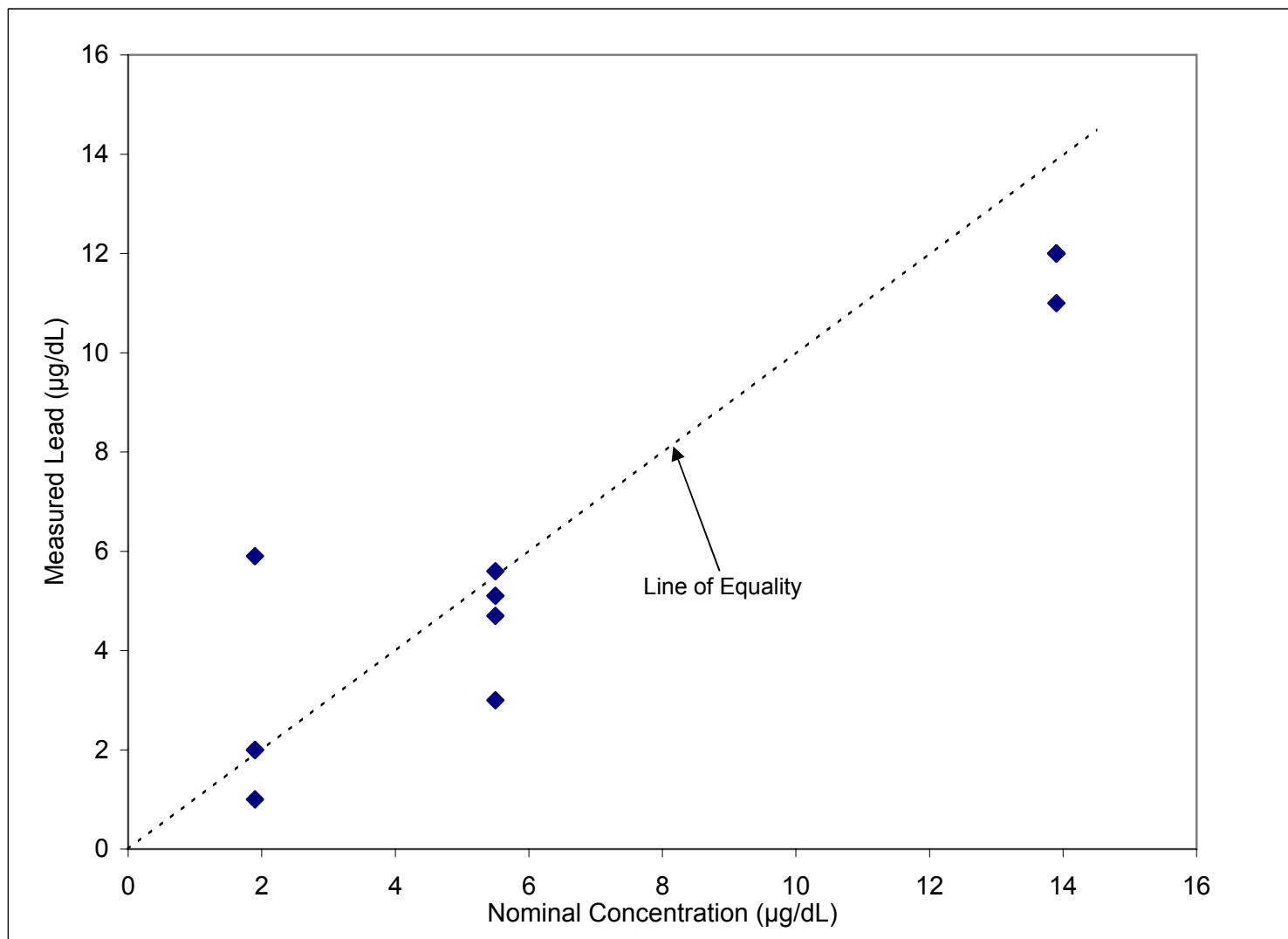
The relative bioavailability (RBA) of arsenic in the test soil compared to that in sodium arsenate was calculated as follows:

$$RBA = \frac{UEF(test\ soil)}{UEF(sodium\ arsenate)}$$

Results are summarized in Table D-3. The arsenic RBA estimates are approximately 14% for the test material. These values are much lower than the default value range of 80%-100% for arsenic in soil that is usually employed when reliable site-specific data are lacking. This indicates that the arsenic in this material is not as well absorbed as soluble arsenic. This relative bioavailability estimate may be used to improve accuracy and decrease uncertainty in estimating human health risks from exposure to this site-specific test material. Supporting detailed information of experimental data used to derive the RBA As for the Deseret soil follows Table D-3.

**Table D-3.** Relative Bioavailable As of the Deseret Soil

### Navy1 Arsenic - Deseret Chemical Depot
### RBA Estimates, Outliers Excluded

| Measurement Endpoint | Estimated RBA (90% Confidence Interval) |
|---|---|
| Days 6/7 | 0.13 (0.11 - 0.15) |
| Days 9/10 | 0.13 (0.12 - 0.14) |
| Days 12/13 | 0.14 (0.13 - 0.16) |
| All Days | 0.14 (0.13 - 0.15) |

**Table D-4 Arsenic - Deseret Chemical Depot**
**RBA Estimates, Outliers Excluded**

| Measurement Endpoint | Estimated RBA (90% Confidence Interval) |
|---|---|
| Days 6/7 | 0.13 (0.11 - 0.15) |
| Days 9/10 | 0.13 (0.12 - 0.14) |
| Days 12/13 | 0.14 (0.13 - 0.16) |
| All Days | 0.14 (0.13 - 0.15) |

2_Navy1 RBA Calcs_outliers.xls (RBA Table)

**TABLE D-5  DOSING PROTOCOL**

| Group | Number of Animals | Dose Material Administered | Arsenic Dose (µg/kg-day) | |
|---|---|---|---|---|
| | | | Target | Actual [a] |
| 1 | 5 | Sodium Arsenate | 25 | 27.6 |
| 2 | 5 | Sodium Arsenate | 50 | 55.8 |
| 3 | 5 | Sodium Arsenate | 100 | 109.8 |
| 4 | 5 | Test Material 1 | 40 | 44.8 |
| 5 | 5 | Test Material 1 | 80 | 87.6 |
| 6 | 5 | Test Material 1 | 160 | 172.9 |
| 7 | 3 | Control | 0 | 0.0 |

[a] Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0-14 for each animal and each group. Actual dose for Group 5 excludes the doses administered to pig number 127, which was euthanized on day 11.

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were held constant based on a body weight of 13.9 kg, the expected mean weight during the exposure interval (14 days). Actual mean body weight across all animals during the exposure interval was 12.9 kg.

4_Navy1 Doses.xls (Tbl2-1)

# TABLE D-6  TYPICAL FEED COMPOSITION

**TestDiet 5TXP:  Porcine Grower Purified Diet with Low Lead[1]**

## INGREDIENTS

| Ingredient | % | Ingredient | % |
|---|---|---|---|
| Corn Starch, % | 25.2 | Potassium Phosphate, % | 0.87 |
| Sucrose, % | 20.9648 | Calcium Carbonate, % | 0.7487 |
| Glucose, % | 16 | Salt, % | 0.501 |
| Soy Protein Isolate, % | 14.9899 | Magnesium Sulfate, % | 0.1245 |
| Casein - Vitamin Free, % | 8.5 | DL-Methionine, % | 0.0762 |
| Powdered Cellulose, % | 6.7208 | Choline Chloride, % | 0.0586 |
| Corn Oil, % | 3.4046 | Vitamin/Mineral Premix, % | 0.0577 |
| Dicalcium Phosphate, % | 1.7399 | Sodium Selenite, % | 0.0433 |

## NUTRITIONAL PROFILE[2]

| Protein, % | 21 | Fat, % | 3.5 |
|---|---|---|---|
| Arginine, % | 1.42 | Cholesterol, ppm | 0 |
| Histidine, % | 0.61 | Linoleic Acid, % | 1.95 |
| Isoleucine, % | 1.14 | Linolenic Acid, % | 0.03 |
| Leucine, % | 1.95 | Arachidonic Acid, % | 0 |
| Lysine, % | 1.56 | Omega-3 Fatty Acids, % | 0.03 |
| Methionine, % | 0.49 | Total Saturated Fatty Acids, % | 0.43 |
| Cystine, % | 0.23 | Total Monounsaturated Fatty Acids, % | 0.82 |
| Phenylalanine, % | 1.22 | Polyunsaturated Fatty Acids, % | 1.98 |
| Tyrosine, % | 1.03 | | |
| Threonine, % | 0.88 | | |
| Tryptophan, % | 0.32 | Fiber (max), % | 6.8 |
| Valine, % | 1.16 | | |
| Alanine, % | 0.95 | Carbohydrates, % | 62.2 |
| Aspartic Acid, % | 2.33 | | |
| Glutamic Acid, % | 4.96 | Energy (kcal/g)[3] | 3.62 |
| Glycine, % | 0.79 | From: | kcal  % |
| Proline, % | 1.83 | Protein | 0.84  23.1 |
| Serine, % | 1.25 | Fat (ether extract) | 0.315  8.7 |
| Taurine, % | 0 | Carbohydrates | 2.487  68.3 |

| Minerals | | Vitamins | |
|---|---|---|---|
| Calcium, % | 0.8 | Vitamin A, IU/g | 1.7 |
| Phosphorus, % | 0.72 | Vitamin 0-3 (added), IU/g | 0.2 |
| Phosphorus (available), % | 0.4 | Vitamin E, IU/kg | 11 |
| Potassium, % | 0.27 | Vitamin K (as menadione), ppm | 0.52 |
| Magnesium, % | 0.04 | Thiamin Hydrochloride, ppm | 1 |
| Sodium, % | 0.3 | Ribonavin, ppm | 3.1 |
| Chlorine, % | 0.31 | Niacin, ppm | 13 |
| Fluorine, ppm | 0 | Pantothenic Acid, ppm | 9 |
| Iron, ppm | 82 | Folic Acid, ppm | 0.3 |
| Zinc, ppm | 84 | Pyridoxine, ppm | 1.7 |
| Manganese, ppm | 3 | Biotin, ppm | 0.1 |
| Copper, ppm | 4.9 | Vitamin B-12, mcg/kg | 15 |
| Cobalt, ppm | 0.1 | Choline Chloride, ppm | 410 |
| Iodine, ppm | 0.15 | Ascorbic Acid, ppm | 0 |
| Chromium, ppm | 0 | | |
| Molybdenum, ppm | 0.01 | | |
| Selenium, ppm | 0.26 | | |

**FOOTNOTES**

[1] This special purified diet was originally developed for lead RBA studies.

[2] Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly. Nutrients expressed as percent of ration on an As Fed basis except where otherwise indicated.

[3] Energy (kcal/gm) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9,4 kcal/gm respectively.

Table 2-2_Feed.xls (PURINA)

FIGURE D-5  BODY WEIGHT GAIN

1_Navy1 Weights.xls (Fig2-1_group weights)
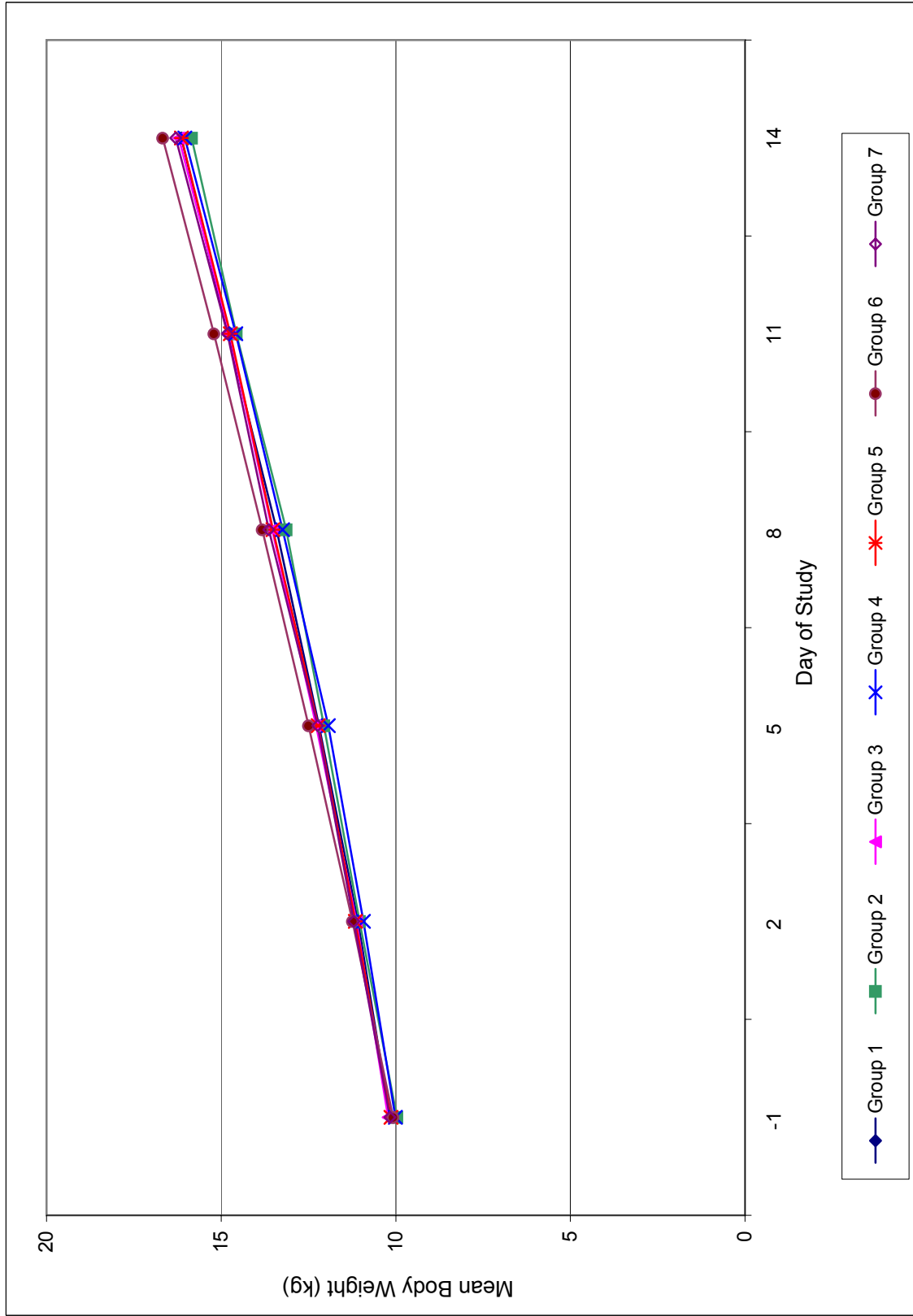
**FIGURE D-6  URINARY ARSENIC BLIND DUPLICATES**

Line of Equality

Primary Analysis (ng/mL)

Duplicate Analysis (ng/mL)

4_Navy1 Lab QC analysis.xls (Fig2-2_BlindDupes)T
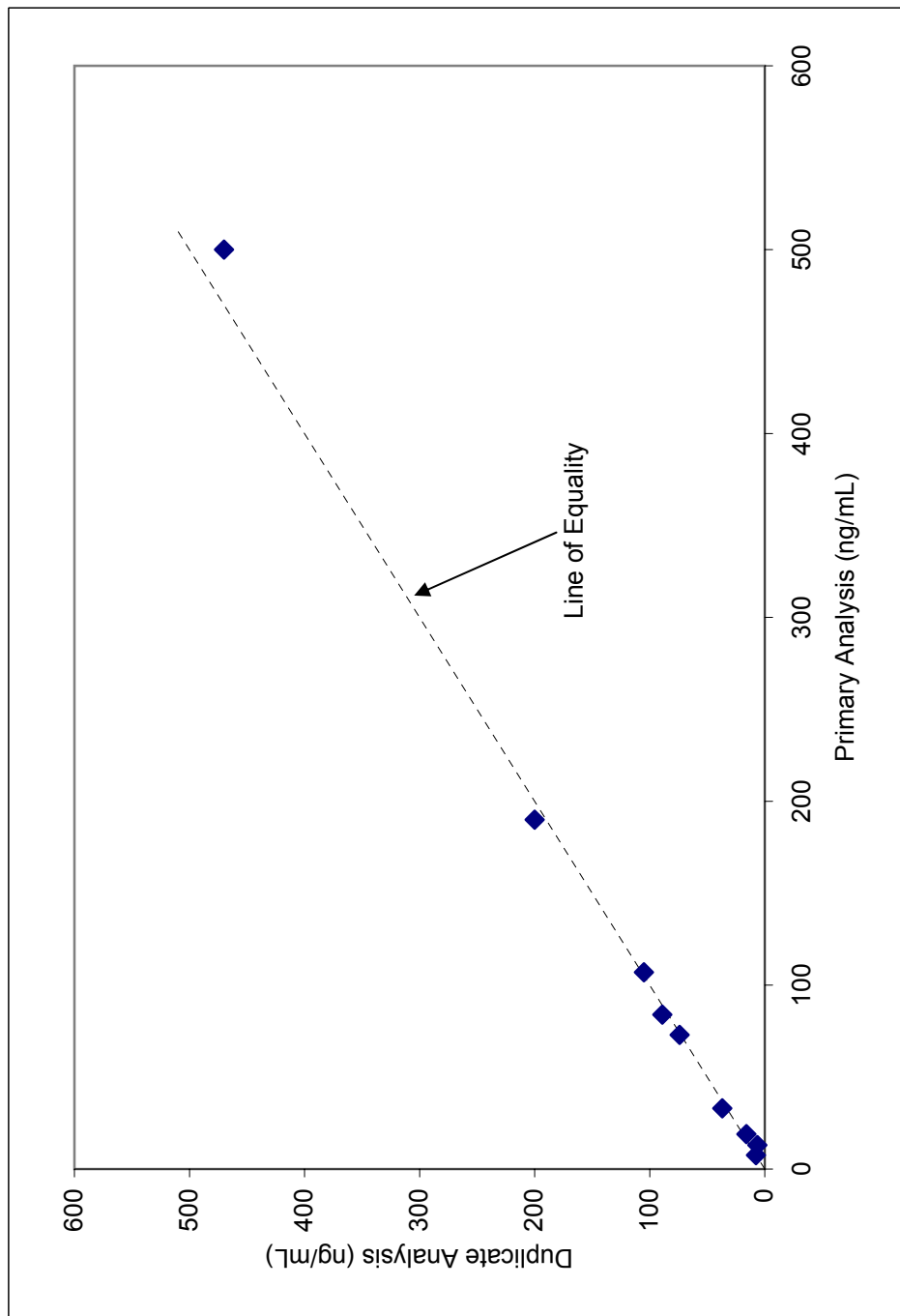
# FIGURE D-7  PERFORMANCE EVALUATION SAMPLES
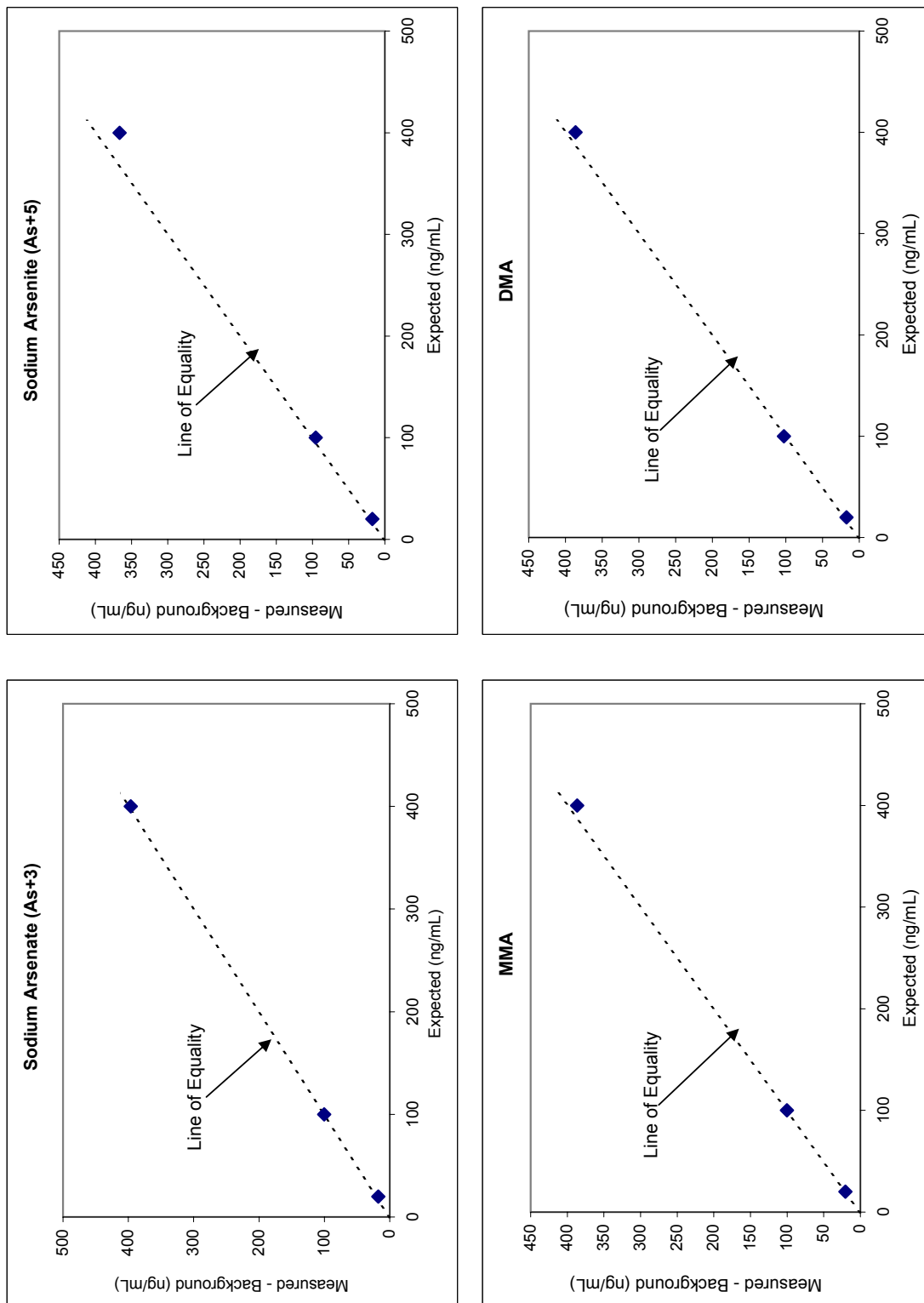


Note:  The MMA result of 103 ng/mL was intended to be a sample of the 400 ng/mL stock solution.  Based on the result of 103 ng/mL, it is presumed that it was actually from the 100 ng/mL stock solution, not 400.  However, this change was not documented so is not known for certain which stock solution was used for this sample.

4_Navy1 Lab QC analysis.xls (Fig2-3_PE)

**Figure D-8. Conceptual Model for Arsenic Toxicokinetics**
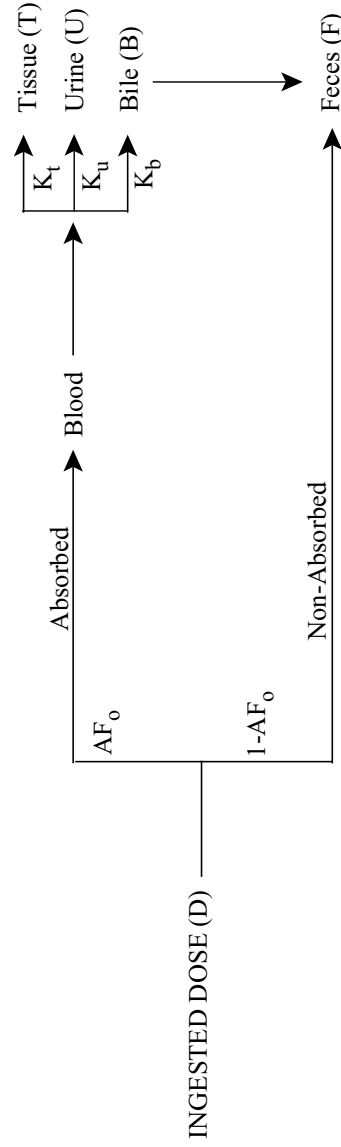


INGESTED DOSE (D)

$AF_o$ — Absorbed → Blood → $K_t$ → Tissue (T)

Blood → $K_u$ → Urine (U)

Blood → $K_b$ → Bile (B) → Feces (F)

$1-AF_o$ — Non-Absorbed → Feces (F)

where:

$D$ = Ingested dose (ug)

$AF_o$ = Oral Absorption Fraction

$K_t$ = Fraction of absorbed arsenic which is retained in tissues

$K_u$ = Fraction of absorbed arsenic which is excreted in urine

$K_b$ = Fraction of absorbed arsenic which is excreted in the bile

BASIC EQUATIONS:

Amount Absorbed (ug) $= D \times AF_o$

Amount Excreted (ug) $= $ Amount absorbed $\times K_u$

$= D \times AF_o \times K_u$

Urinary Excretion Fraction (UEF) $= $ Amount excreted / Amount Ingested

$= (D \times AF_o \times K_u) / D$

$= AF_o \times K_u$

Relative Bioavailability (x vs. y) $= UEF(x) / UEF(y)$

$= (AF_o(x) \times K_u) / (AF_o(y) \times K_u)$

$= AF_o(x) / AF_o(y)$

Fig 3-1_Toxicokinetics.wpd

FIGURE D-9  URINARY ARSENIC VARIANCE MODEL

Legend:
◇ Model - Controls
○ Model - Sodium Arsenate
△ Model - Test Materials
◆ Navy1 - Controls
● Navy1 - Sodium Arsenate
▲ Navy1 - Test Materials

x-axis: ln(Group Mean Response)
y-axis: ln(Group Variance)

3_Navy1 Variance.xls (Fig 4-1)

D-19
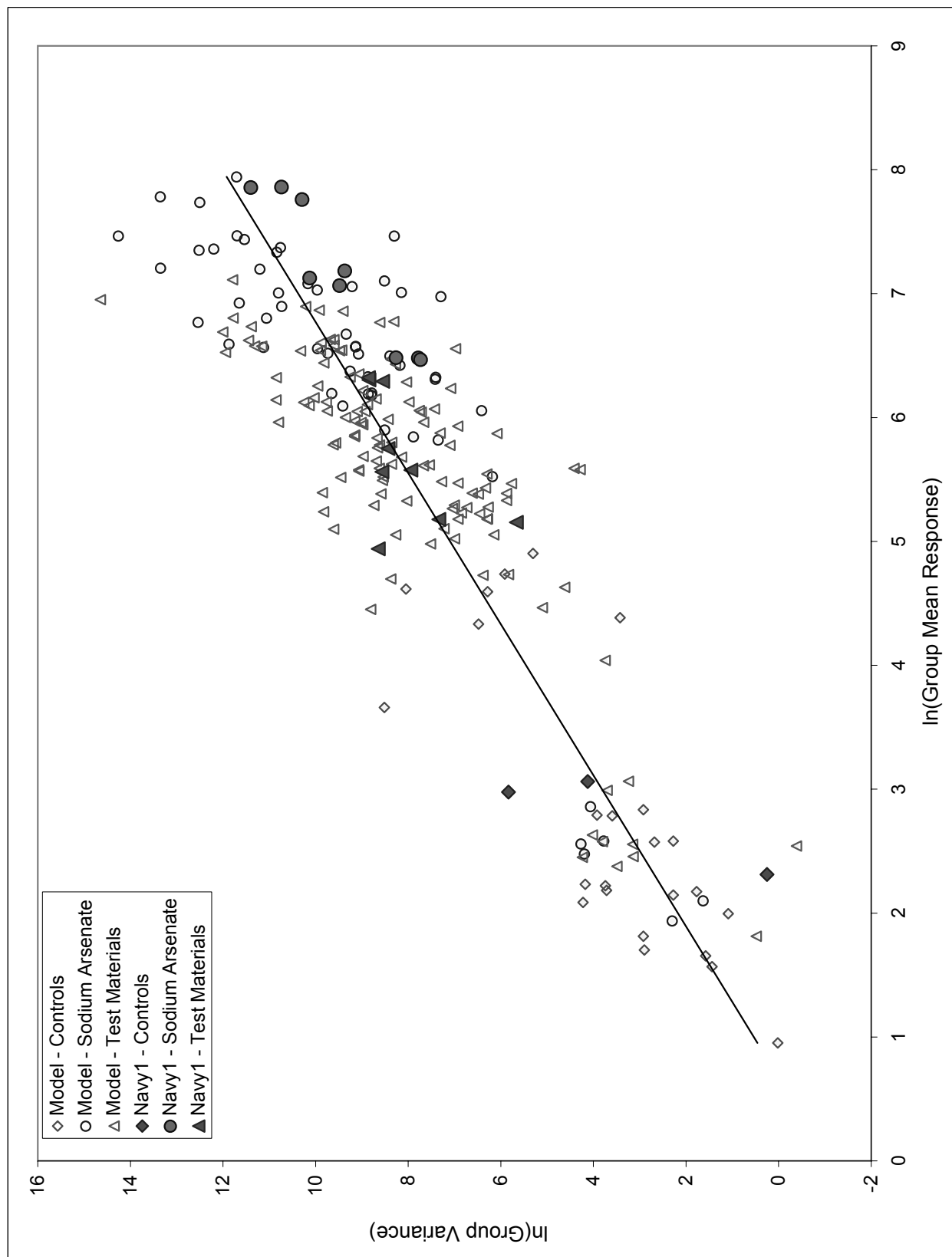
# FIGURE D-10 URINARY EXCRETION OF ARSENIC: Days 6/7 (All Data)

**Reference Material (Sodium Arsenate)**
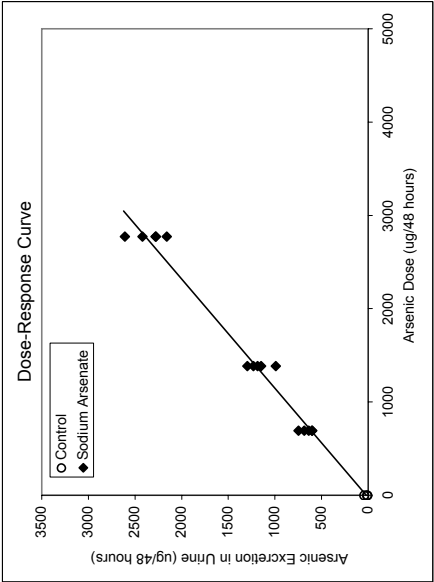
**Test Material 1 (Deseret Chemical Depot)**

*Test Material: Magnified Scale*

Dose-Response Curve

Arsenic Excretion in Urine (ug/48 hours)

Arsenic Dose (ug/48 hours)

Y-axis magnified to show detail

Residual Plot

Standardized Weighted Residual

SQRT(W) * Dose

o Control
◆ Sodium Arsenate

o Control
△ Test Material 1
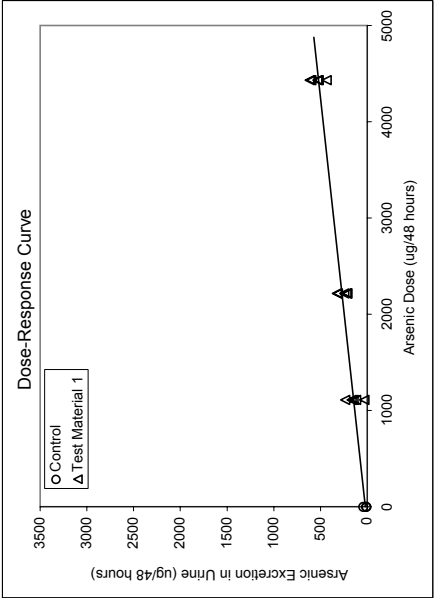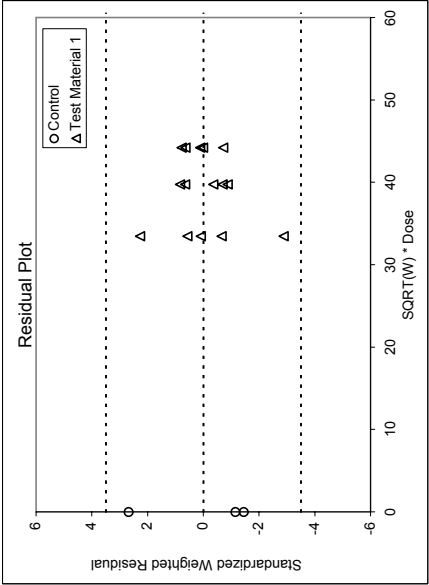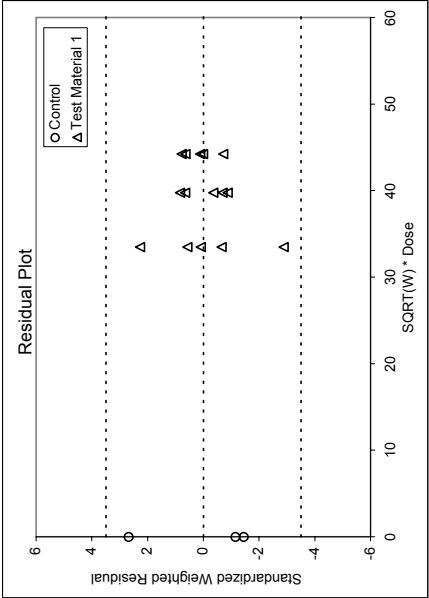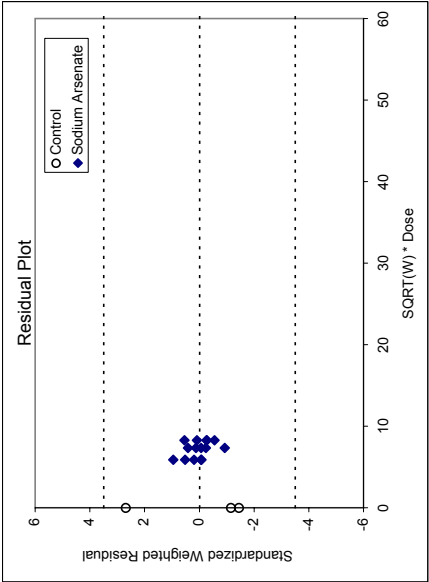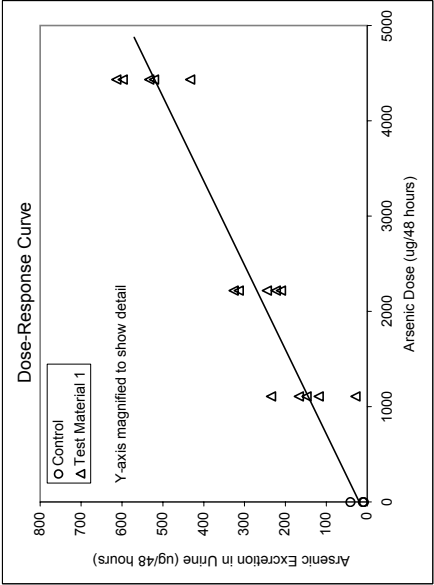
## Summary of Fitting[a]

| Parameter | Estimate | Standard Error |
|---|---|---|
| a | 19.5 | 4.7 |
| $b_r$ | 0.85 | 0.04 |
| $b_{t1}$ | 0.11 | 0.01 |
| $b_{t2}$ | -- | -- |
| Covariance ($b_r, b_{t1}$) | 0.0197 | -- |
| Covariance ($b_r, b_{t2}$) | -- | -- |
| Degrees of Freedom | 31 | |

[a] $y = a + b_r * x_r + b_{t1} * x_{t1} + b_{t2} * x_{t2}$

*where* r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

## ANOVA

| Source | SSE | DF | MSE |
|---|---|---|---|
| Fit | 831.63 | 2 | 415.82 |
| Error | 46.91 | 30 | 1.56 |
| Total | 878.54 | 32 | 27.45 |

| Statistic | Estimate |
|---|---|
| F | 265.913 |
| p | < 0.001 |
| Adjusted $R^2$ | 0.9430 |

## RBA and Uncertainty

| | Test Material 1 | Test Material 2 |
|---|---|---|
| RBA | 0.13 | -- |
| Lower bound[b] | 0.11 | -- |
| Upper bound[b] | 0.15 | -- |
| Standard Error[b] | 0.012 | -- |

[b] Calculated using Fieller's theorem

2_Navy1 RBA Calcs_all data.xls (Graph_1)

D-20

# FIGURE D-11  URINARY EXCRETION OF ARSENIC:  Days 9/10 (All Data)

## Reference Material (Sodium Arsenate)

**Dose-Response Curve**

Arsenic Excretion in Urine (ug/48 hours) vs Arsenic Dose (ug/48 hours)

○ Control   ◆ Sodium Arsenate

**Residual Plot**

Standardized Weighted Residual vs SQRT(W) * Dose

○ Control   ◆ Sodium Arsenate

## Test Material 1 (Deseret Chemical Depot)

**Dose-Response Curve**

Arsenic Excretion in Urine (ug/48 hours) vs Arsenic Dose (ug/48 hours)

○ Control   △ Test Material 1

**Residual Plot**

Standardized Weighted Residual vs SQRT(W) * Dose

○ Control   △ Test Material 1

## Test Material: *Magnified Scale*

**Dose-Response Curve**

Y-axis magnified to show detail

Arsenic Excretion in Urine (ug/48 hours) vs Arsenic Dose (ug/48 hours)

○ Control   △ Test Material 1

**Residual Plot**

Standardized Weighted Residual vs SQRT(W) * Dose

○ Control   △ Test Material 1

### Summary of Fitting[a]

| Parameter | Estimate | SE |
|---|---|---|
| a | 21.9 | 3.3 |
| $b_r$ | 0.91 | 0.03 |
| $b_{t1}$ | 0.12 | 0.01 |
| $b_{t2}$ | -- | -- |
| Covariance ($b_r, b_{t1}$) | 0.0202 | |
| Covariance ($b_r, b_{t2}$) | -- | |
| Degrees of Freedom | 31 | |

[a] $y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1} + b_{t2} \cdot x_{t2}$

*where* r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

### ANOVA

| Source | SSE | DF | MSE |
|---|---|---|---|
| Fit | 846.04 | 2 | 423.02 |
| Error | 19.99 | 30 | 0.67 |
| Total | 866.03 | 32 | 27.06 |

| Statistic | Estimate |
|---|---|
| F | 634.759 |
| p | < 0.001 |
| Adjusted $R^2$ | 0.9754 |

### RBA and Uncertainty

| | Test Material 1 | Test Material 2 |
|---|---|---|
| RBA | 0.13 | -- |
| Lower bound[b] | 0.12 | -- |
| Upper bound[b] | 0.14 | -- |
| Standard Error[b] | 0.008 | -- |

[b] Calculated using Fieller's theorem

D-21

2_Navy1 RBA Calcs_all data.xls (Graph_2)

*Test Material:  Magnified Scale*

**Reference Material (Sodium Arsenate)**
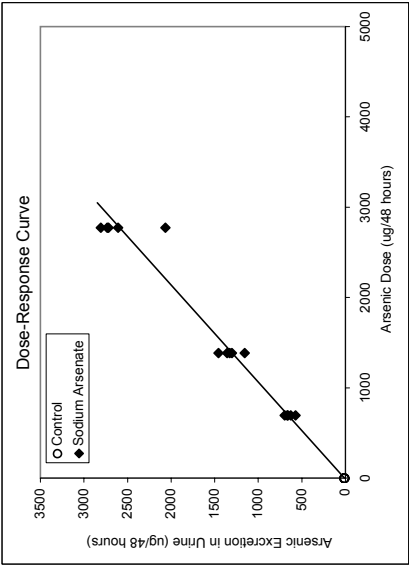
**Test Material 1 (Deseret Chemical Depot)**
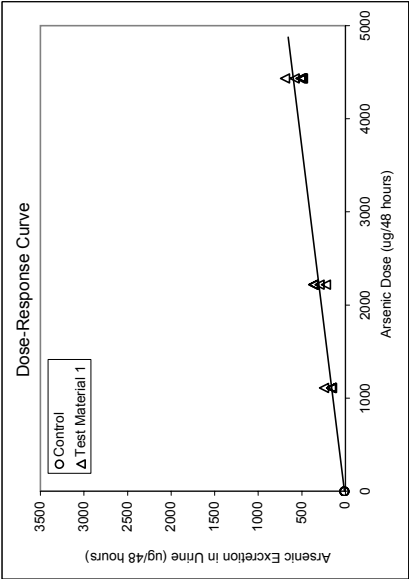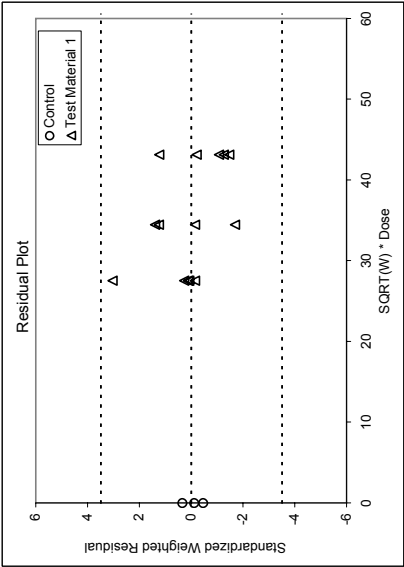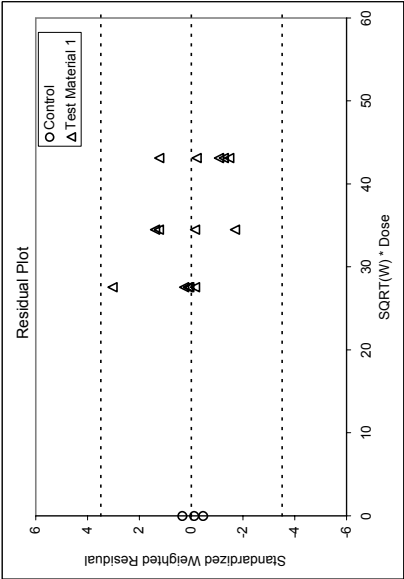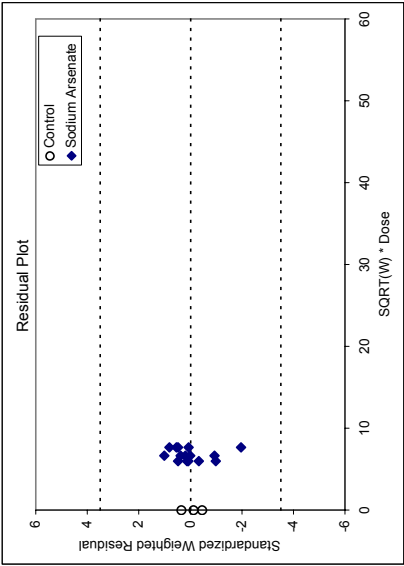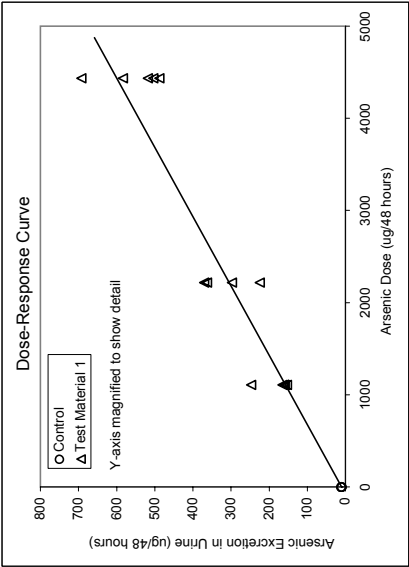


**Summary of Fitting**[a]

| Parameter | Estimate | SE |
|---|---|---|
| a | 10.3 | 1.7 |
| $b_r$ | 0.93 | 0.03 |
| $b_{t1}$ | 0.13 | 0.01 |
| $b_{t2}$ | -- | -- |
| Covariance $(b_r, b_{t1})$ | 0.0055 | |
| Covariance $(b_r, b_{t2})$ | -- | |
| Degrees of Freedom | 30 | |

[a] $y = a + b_r*x_r + b_{t1}*x_{t1} + b_{t2}*x_{t2}$

*where* r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

**ANOVA**

| Source | SSE | DF | MSE |
|---|---|---|---|
| Fit | 903.83 | 2 | 451.92 |
| Error | 16.71 | 29 | 0.58 |
| Total | 920.54 | 31 | 29.69 |

| Statistic | Estimate |
|---|---|
| F | 784.248 |
| p | < 0.001 |
| Adjusted $R^2$ | 0.9806 |

**RBA and Uncertainty**

| | Test Material 1 | Test Material 2 |
|---|---|---|
| RBA | 0.14 | -- |
| Lower bound[b] | 0.13 | -- |
| Upper bound[b] | 0.16 | -- |
| Standard Error[b] | 0.008 | -- |

[b] Calculated using Fieller's theorem

D-22

2_Navy1 RBA Calcs_all data.xls (Graph_3)

# FIGURE D-13 URINARY EXCRETION OF ARSENIC:  All Days (All Data)
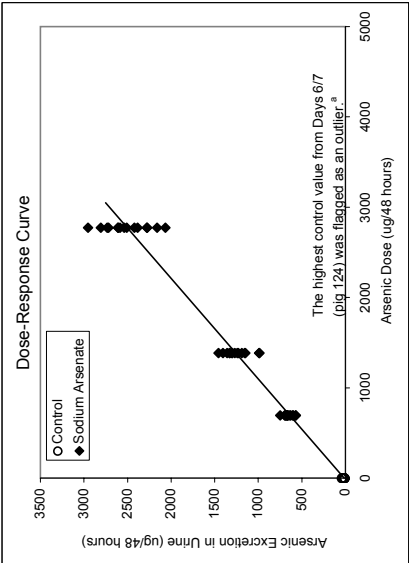
## *Test Material:  Magnified Scale*

## Reference Material (Sodium Arsenate)

## Test Material 1 (Deseret Chemical Depot)
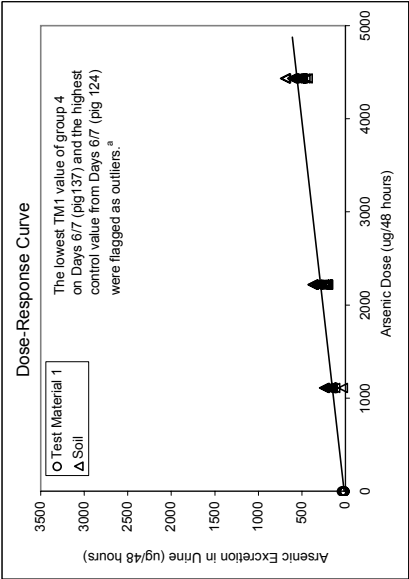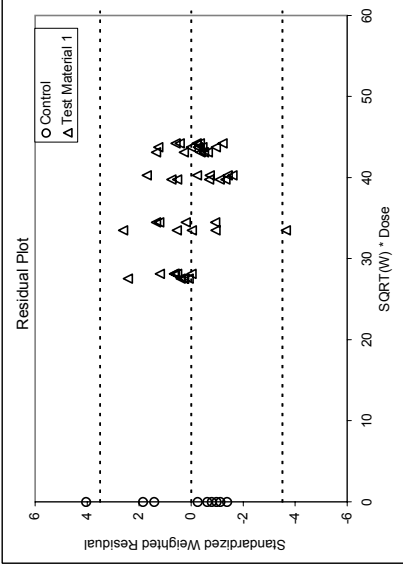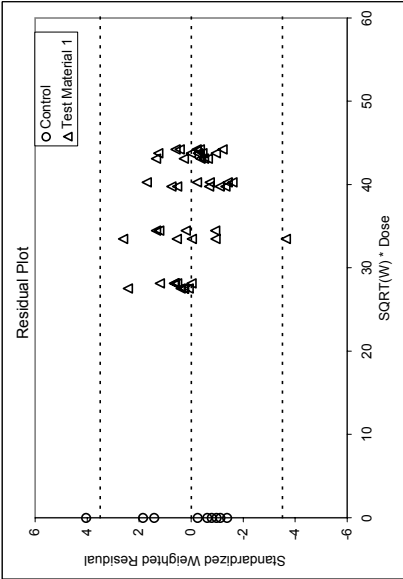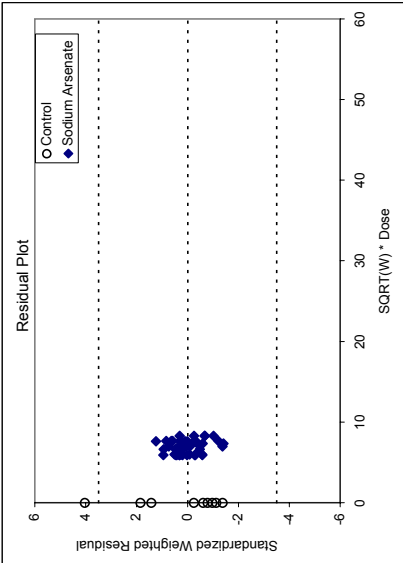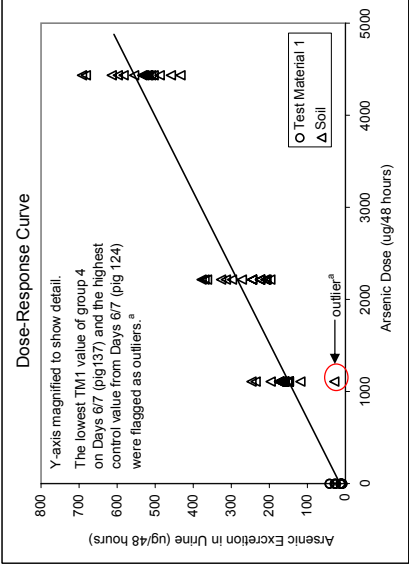


[a] Note that the data from this figure were refitted with the outliers excluded (see Figure 4-5b); these outliers were excluded from the final evaluation for arsenic RBA.

### Summary of Fitting[b]

| Parameter | Estimate | SE |
|---|---|---|
| a | 14.3 | 1.7 |
| $b_r$ | 0.90 | 0.02 |
| $b_{t1}$ | 0.12 | 0.00 |
| $b_{t2}$ | -- | -- |
| Covariance ($b_r, b_{t1}$) | 0.0113 | |
| Covariance ($b_r, b_{t2}$) | -- | |
| Degrees of Freedom | 96 | |

[b] $y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1} + b_{t2} \cdot x_{t2}$

*where* r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

### ANOVA

| Source | SSE | DF | MSE |
|---|---|---|---|
| Fit | 2627.64 | 2 | 1313.82 |
| Error | 96.86 | 95 | 1.02 |
| Total | 2724.49 | 97 | 28.09 |

| Statistic | Estimate |
|---|---|
| F | 1288.642 |
| p | <0.001 |
| Adjusted $R^2$ | 0.9637 |

### RBA and Uncertainty

| | Test Material 1 | Test Material 2 |
|---|---|---|
| RBA | 0.14 | -- |
| Lower bound[c] | 0.13 | -- |
| Upper bound[c] | 0.14 | -- |
| Standard Error[c] | 0.006 | |

[c] Calculated using Fieller's theorem

2_Navy1 RBA Calcs_all data.xls (Graph_All)

D-23

# FIGURE D-14 URINARY EXCRETION OF ARSENIC: All Days (Outliers Excluded)

**Reference Material (Sodium Arsenate)**
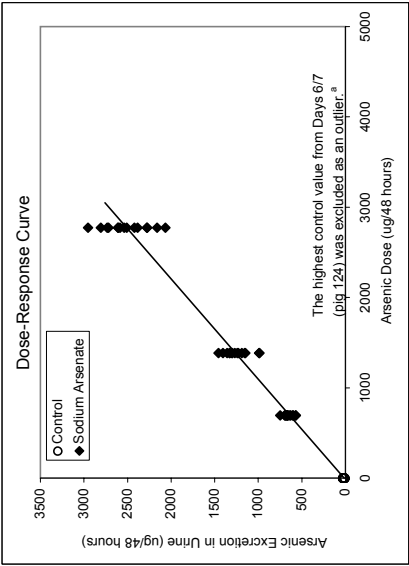
**Test Material 1 (Deseret Chemical Depot)**
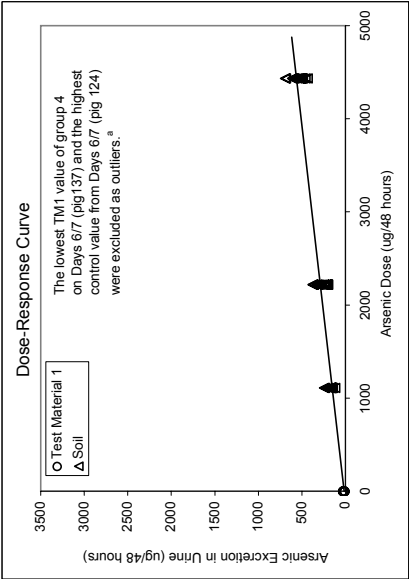
*Test Material: Magnified Scale*
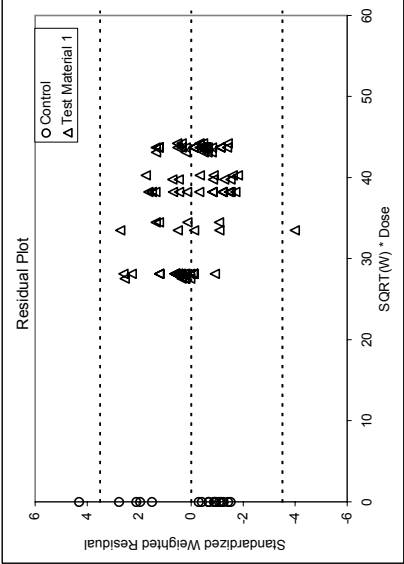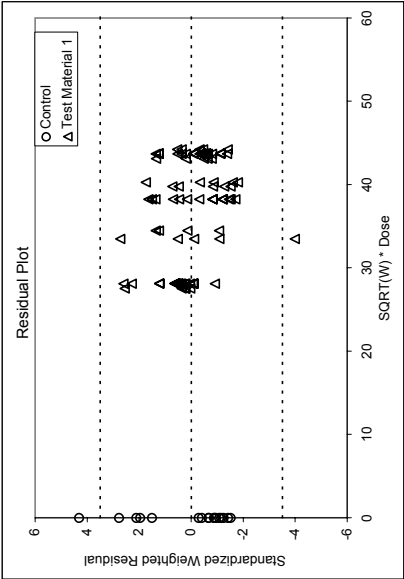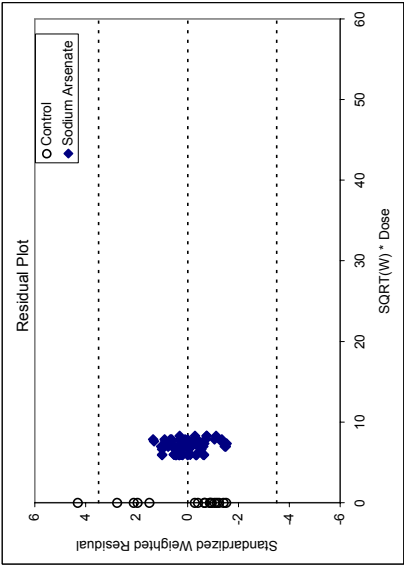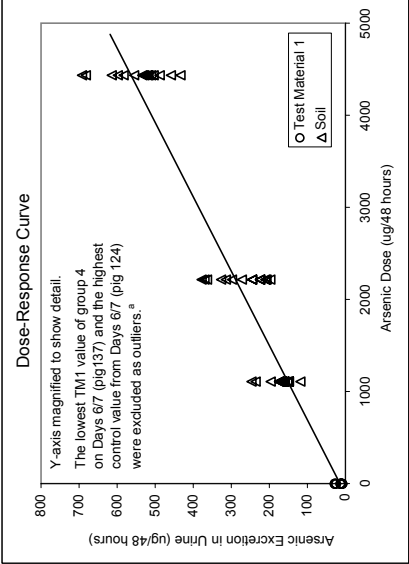


[a] The outliers were identified in the initial fitting (see Figure 4-5a); the data are plotted here (Figure 4-5b) with the outliers excluded. These results, with the outliers excluded, were used in the final evaluation for arsenic RBA.

**Summary of Fitting[b]**

| Parameter | Estimate | SE |
|---|---|---|
| a | 14.4 | 1.5 |
| $b_r$ | 0.90 | 0.02 |
| $b_{t1}$ | 0.12 | 0.00 |
| $b_{t2}$ | -- | -- |
| Covariance ($b_r, b_{t1}$) | 0.0111 | |
| Covariance ($b_r, b_{t2}$) | -- | |
| Degrees of Freedom | 192 | |

[b] $y = a + b_r*x_r + b_{t1}*x_{t1} + b_{t2}*x_{t2}$

*where* r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

**ANOVA**

| Source | SSE | DF | MSE |
|---|---|---|---|
| Fit | 5296.37 | 2 | 2648.18 |
| Error | 83.47 | 93 | 0.90 |
| Total | 5379.84 | 95 | 56.63 |

| Statistic | Estimate |
|---|---|
| F | 2950.448 |
| p | < 0.001 |
| Adjusted $R^2$ | 0.9842 |

**RBA and Uncertainty**

| | Test Material 1 | Test Material 2 |
|---|---|---|
| RBA | 0.14 | -- |
| Lower bound[c] | 0.13 | -- |
| Upper bound[c] | 0.15 | -- |
| Standard Error[c] | 0.005 | -- |

[c] Calculated using Fieller's theorem

D-24

2_Navy1 RBA Calcs_outliers.xls (Graph_All)

**TABLE D-7  SCHEDULE**

| Study Day | Day | Date | Feed Special Diet | Cull Pigs/ Assign Dose Group | Weigh | Dose Preparation | Dose Administration | Urine Collection[a] | Sacrifice/ Necropsy |
|---|---|---|---|---|---|---|---|---|---|
| -6 | Tuesday | 09/26/06 | | | | | | | |
| -5 | Wednesday | 09/27/06 | | X | X | | | | |
| -4 | Thursday | 09/28/06 | transition | | | | | | |
| -3 | Friday | 09/29/06 | transition | | | | | | |
| -2 | Saturday | 09/30/06 | transition | | | | | | |
| -1 | Sunday | 10/01/06 | transition | | X | X | | | |
| 0 | Monday | 10/02/06 | X | | | | X | | |
| 1 | Tuesday | 10/03/06 | X | | | | X | | |
| 2 | Wednesday | 10/04/06 | X | | X | | X | | |
| 3 | Thursday | 10/05/06 | X | | | X | X | | |
| 4 | Friday | 10/06/06 | X | | | | X | | |
| 5 | Saturday | 10/07/06 | X | | X | | X | | |
| 6 | Sunday | 10/08/06 | X | | | | X | ↕ U-1 | |
| 7 | Monday | 10/09/06 | X | | | | X | | |
| 8 | Tuesday | 10/10/06 | X | | X | X[b] | X | | |
| 9 | Wednesday | 10/11/06 | X | | | | X | ↕ U-2 | |
| 10 | Thursday | 10/12/06 | X | | | | X | | |
| 11 | Friday | 10/13/06 | X | | X | | X | | |
| 12 | Saturday | 10/14/06 | X | | | | X | ↕ U-3 | |
| 13 | Sunday | 10/15/06 | X | | | | X | | |
| 14 | Monday | 10/16/06 | X | | X | | | | X |

[a] Urine was collected over a period of 48 hours.

[a] The original schedule called for dose preparation to occur on days -1, 3, 7, and 11.  However, due to scheduling conflicts, the day 7 and 11 preparations were replaced with a single preparation on day 8.

**TABLE D-8  GROUP ASSIGNMENTS**

| Pig Number | Dose Group | Material Administered | Target Dose of Arsenic (µg/kg-day) |
|---|---|---|---|
| 101<br>103<br>110<br>114<br>132 | 1 | Sodium Arsenate | 25 |
| 105<br>107<br>121<br>130<br>133 | 2 | Sodium Arsenate | 50 |
| 104<br>113<br>125<br>126<br>138 | 3 | Sodium Arsenate | 100 |
| 102<br>116<br>129<br>134<br>137 | 4 | Test Material 1 | 40 |
| 106<br>117<br>118<br>122<br>127 | 5 | Test Material 1 | 80 |
| 108<br>120<br>123<br>128<br>131 | 6 | Test Material 1 | 160 |
| 109<br>124<br>135 | 7 | Control | 0 |

Navy1_Appendix A.xls (A-2_Groups)

**TABLE D-9  BODY WEIGHTS AND ACTUAL ADMINISTERED DOSES, BY DAY**

Body weights were measured on days -1, 2, 5, 8, 11, and 14.  Weights for other days are estimated, based on linear interpolation between measured values.

| Group | Pig # | Day -1 BW (kg) | Day -1 As Dose (µg/Kg-d) | Day 0 BW (kg) | Day 0 As Dose (µg/Kg-d) | Day 1 BW (kg) | Day 1 As Dose (µg/Kg-d) | Day 2 BW (kg) | Day 2 As Dose (µg/Kg-d) | Day 3 BW (kg) | Day 3 As Dose (µg/Kg-d) | Day 4 BW (kg) | Day 4 As Dose (µg/Kg-d) | Day 5 BW (kg) | Day 5 As Dose (µg/Kg-d) | Day 6 BW (kg) | Day 6 As Dose (µg/Kg-d) | Day 7 BW (kg) | Day 7 As Dose (µg/Kg-d) | Day 8 BW (kg) | Day 8 As Dose (µg/Kg-d) | Day 9 BW (kg) | Day 9 As Dose (µg/Kg-d) | Day 10 BW (kg) | Day 10 As Dose (µg/Kg-d) | Day 11 BW (kg) | Day 11 As Dose (µg/Kg-d) | Day 12 BW (kg) | Day 12 As Dose (µg/Kg-d) | Day 13 BW (kg) | Day 13 As Dose (µg/Kg-d) | Day 14 BW (kg) | Day 14 As Dose (µg/Kg-d) | Days 0-14 Mean As Dose (µg/Kg-d) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 101 | 9.8 | 0.00 | 10.1 | 34.18 | 10.5 | 33.09 | 10.8 | 32.07 | 11.2 | 30.93 | 11.6 | 29.86 | 12.0 | 28.86 | 12.4 | 28.01 | 12.7 | 27.20 | 13.1 | 26.44 | 13.6 | 25.47 | 14.1 | 24.56 | 14.6 | 23.72 | 15.0 | 23.07 | 15.4 | 22.44 | 15.9 | 21.85 | 28.17 |
| 1 | 103 | 10.2 | 0.00 | 10.6 | 32.83 | 10.9 | 31.78 | 11.3 | 30.79 | 11.6 | 29.73 | 12.1 | 28.74 | 12.5 | 27.82 | 12.9 | 26.82 | 13.4 | 25.88 | 13.9 | 25.01 | 14.3 | 24.16 | 14.8 | 23.38 | 15.3 | 22.64 | 15.7 | 22.04 | 16.1 | 21.47 | 16.6 | 20.93 | 26.99 |
| 1 | 110 | 10.2 | 0.00 | 10.5 | 32.93 | 10.9 | 31.83 | 11.3 | 30.79 | 11.6 | 29.86 | 12.0 | 28.98 | 12.3 | 28.16 | 12.7 | 27.20 | 13.2 | 26.31 | 13.6 | 25.47 | 14.1 | 24.59 | 14.6 | 23.78 | 15.1 | 23.01 | 15.5 | 22.35 | 16.0 | 21.72 | 16.4 | 21.12 | 27.23 |
| 1 | 114 | 10.0 | 0.00 | 10.2 | 34.07 | 10.4 | 33.36 | 10.6 | 32.68 | 11.0 | 31.49 | 11.4 | 30.38 | 11.8 | 29.35 | 12.1 | 28.55 | 12.5 | 27.78 | 12.8 | 27.06 | 13.3 | 26.14 | 13.7 | 25.28 | 14.2 | 24.48 | 14.6 | 23.75 | 15.0 | 23.07 | 15.5 | 22.42 | 28.66 |
| 1 | 132 | 10.8 | 0.00 | 11.0 | 31.49 | 11.3 | 30.79 | 11.5 | 30.12 | 11.8 | 29.31 | 12.1 | 28.55 | 12.5 | 27.82 | 12.9 | 26.92 | 13.3 | 26.08 | 13.7 | 25.28 | 14.1 | 24.54 | 14.5 | 23.83 | 15.0 | 23.17 | 15.5 | 22.42 | 16.0 | 21.72 | 16.5 | 21.06 | 26.79 |
| 2 | 105 | 9.8 | 0.00 | 10.2 | 68.25 | 10.6 | 65.66 | 11.0 | 63.26 | 11.2 | 61.85 | 11.5 | 60.50 | 11.7 | 59.21 | 12.0 | 57.81 | 12.3 | 56.47 | 12.6 | 55.20 | 13.1 | 53.01 | 13.6 | 51.00 | 14.1 | 49.13 | 14.5 | 47.77 | 14.9 | 46.49 | 15.3 | 45.28 | 57.31 |
| 2 | 107 | 9.5 | 0.00 | 9.9 | 69.97 | 10.3 | 67.26 | 10.7 | 64.74 | 11.1 | 62.41 | 11.5 | 60.24 | 11.9 | 58.21 | 12.3 | 56.24 | 12.7 | 54.40 | 13.2 | 52.68 | 13.7 | 50.69 | 14.2 | 48.84 | 14.7 | 47.12 | 15.0 | 46.08 | 15.4 | 45.08 | 15.7 | 44.12 | 56.79 |
| 2 | 121 | 9.8 | 0.00 | 10.2 | 67.69 | 10.7 | 64.94 | 11.1 | 59.29 | 11.4 | 60.59 | 11.8 | 58.87 | 12.1 | 57.25 | 12.4 | 56.02 | 12.7 | 54.83 | 12.9 | 53.70 | 13.4 | 51.89 | 13.8 | 50.20 | 14.3 | 48.61 | 14.7 | 47.23 | 15.1 | 45.93 | 15.5 | 44.69 | 55.92 |
| 2 | 130 | 10.2 | 0.00 | 10.5 | 65.87 | 10.8 | 63.94 | 11.2 | 62.13 | 11.5 | 60.24 | 11.9 | 58.46 | 12.2 | 56.78 | 12.6 | 54.98 | 13.0 | 53.29 | 13.4 | 51.70 | 14.0 | 49.66 | 14.5 | 47.77 | 15.1 | 46.03 | 15.5 | 44.60 | 16.0 | 43.25 | 16.5 | 41.98 | 54.77 |
| 2 | 133 | 10.6 | 0.00 | 10.8 | 63.94 | 11.1 | 62.60 | 11.3 | 61.30 | 11.3 | 59.38 | 12.0 | 57.57 | 12.4 | 55.87 | 12.8 | 53.98 | 13.3 | 52.22 | 13.7 | 50.56 | 13.7 | 49.30 | 14.4 | 48.11 | 14.8 | 46.96 | 15.3 | 45.42 | 15.8 | 43.98 | 16.3 | 42.63 | 54.12 |
| 3 | 104 | 10.5 | 0.00 | 10.8 | 128.09 | 11.2 | 123.89 | 11.6 | 119.95 | 12.1 | 114.82 | 12.6 | 110.10 | 13.1 | 105.76 | 13.5 | 103.01 | 13.8 | 100.40 | 14.2 | 97.91 | 14.6 | 95.22 | 15.0 | 92.67 | 15.4 | 90.26 | 15.8 | 87.87 | 16.2 | 85.61 | 16.6 | 83.46 | 105.07 |
| 3 | 113 | 11.2 | 0.00 | 11.4 | 121.35 | 11.7 | 118.58 | 12.0 | 115.94 | 12.3 | 112.79 | 12.6 | 109.81 | 13.0 | 106.98 | 13.3 | 103.91 | 13.7 | 101.01 | 14.1 | 98.26 | 14.5 | 95.66 | 14.9 | 93.19 | 15.3 | 90.85 | 15.8 | 87.69 | 16.4 | 84.74 | 16.9 | 81.98 | 103.59 |
| 3 | 125 | 9.8 | 0.00 | 10.2 | 136.50 | 10.6 | 131.32 | 11.0 | 126.53 | 11.2 | 123.33 | 11.5 | 120.30 | 11.8 | 117.41 | 12.2 | 113.56 | 12.6 | 109.96 | 13.0 | 106.57 | 13.4 | 103.26 | 13.8 | 100.15 | 14.3 | 97.22 | 14.7 | 94.14 | 15.2 | 91.25 | 15.7 | 88.53 | 113.28 |
| 3 | 126 | 9.7 | 0.00 | 10.1 | 137.86 | 10.5 | 132.58 | 10.9 | 127.69 | 11.3 | 122.97 | 11.7 | 118.58 | 12.1 | 114.50 | 12.6 | 109.96 | 13.1 | 105.76 | 13.6 | 101.87 | 14.0 | 99.20 | 14.3 | 96.66 | 14.7 | 94.25 | 15.1 | 91.55 | 15.6 | 89.00 | 16.0 | 86.59 | 111.58 |
| 3 | 138 | 10.1 | 0.00 | 10.2 | 136.05 | 10.3 | 134.95 | 10.4 | 133.86 | 10.7 | 129.28 | 11.1 | 125.00 | 11.5 | 121.00 | 11.9 | 116.26 | 12.4 | 111.88 | 12.9 | 107.82 | 13.3 | 104.04 | 13.8 | 100.52 | 14.3 | 97.22 | 14.8 | 93.61 | 15.4 | 90.26 | 15.9 | 87.14 | 115.59 |
| 4 | 102 | 10.7 | 0.00 | 11.1 | 50.08 | 11.4 | 48.47 | 11.8 | 46.96 | 12.2 | 45.42 | 12.6 | 43.98 | 13.0 | 42.63 | 13.4 | 41.36 | 13.8 | 40.16 | 14.2 | 39.03 | 14.7 | 37.79 | 15.1 | 36.62 | 15.6 | 35.52 | 16.0 | 34.56 | 16.5 | 33.65 | 16.9 | 32.79 | 41.59 |
| 4 | 116 | 9.9 | 0.00 | 10.1 | 55.05 | 10.3 | 53.89 | 10.5 | 52.78 | 10.9 | 51.08 | 11.2 | 49.48 | 11.6 | 47.98 | 12.0 | 46.38 | 12.4 | 44.87 | 12.8 | 43.47 | 13.2 | 42.14 | 13.6 | 40.90 | 14.0 | 39.73 | 14.4 | 38.48 | 14.9 | 37.32 | 15.3 | 36.22 | 46.42 |
| 4 | 129 | 9.7 | 0.00 | 10.1 | 55.14 | 10.5 | 53.03 | 10.9 | 51.08 | 11.1 | 49.85 | 11.4 | 48.68 | 11.7 | 47.57 | 12.1 | 45.67 | 12.6 | 43.92 | 13.1 | 42.30 | 13.5 | 41.10 | 13.9 | 39.97 | 14.3 | 38.89 | 14.8 | 37.49 | 15.3 | 36.18 | 15.9 | 34.96 | 45.49 |
| 4 | 134 | 9.9 | 0.00 | 10.2 | 54.42 | 10.5 | 52.95 | 10.8 | 51.55 | 11.1 | 49.93 | 11.5 | 48.40 | 11.8 | 46.96 | 12.2 | 45.49 | 12.6 | 44.10 | 13.0 | 42.79 | 13.5 | 41.00 | 14.1 | 39.35 | 14.7 | 37.83 | 15.2 | 36.58 | 15.7 | 35.41 | 16.2 | 34.31 | 45.24 |
| 4 | 137 | 10.0 | 0.00 | 10.2 | 54.24 | 10.5 | 52.86 | 10.8 | 51.55 | 11.1 | 50.15 | 11.4 | 48.83 | 11.7 | 47.57 | 12.2 | 45.55 | 12.7 | 43.69 | 13.2 | 41.98 | 13.6 | 40.70 | 14.0 | 39.49 | 14.5 | 38.35 | 15.0 | 37.03 | 15.5 | 35.79 | 16.0 | 34.64 | 45.32 |
| 5 | 106 | 10.1 | 0.00 | 10.4 | 106.23 | 10.8 | 102.47 | 11.2 | 98.96 | 11.5 | 96.24 | 11.8 | 93.66 | 12.2 | 91.22 | 12.5 | 88.43 | 12.9 | 85.81 | 13.3 | 83.34 | 13.6 | 81.50 | 13.9 | 79.74 | 14.2 | 78.05 | 14.6 | 75.83 | 15.0 | 73.73 | 15.5 | 71.74 | 88.97 |
| 5 | 117 | 10.3 | 0.00 | 10.7 | 103.91 | 11.0 | 100.46 | 11.4 | 97.22 | 11.8 | 94.06 | 12.2 | 91.10 | 12.6 | 88.32 | 13.0 | 85.48 | 13.4 | 82.82 | 13.8 | 80.32 | 14.5 | 76.61 | 15.1 | 73.24 | 15.8 | 70.15 | 16.2 | 68.28 | 16.7 | 66.50 | 17.1 | 64.82 | 85.27 |
| 5 | 118 | 10.3 | 0.00 | 10.2 | 108.84 | 10.4 | 106.40 | 10.7 | 104.07 | 11.0 | 100.30 | 11.5 | 96.80 | 11.9 | 93.53 | 12.3 | 89.99 | 12.8 | 86.70 | 13.3 | 83.65 | 13.7 | 80.90 | 14.2 | 78.33 | 14.6 | 75.92 | 15.0 | 74.14 | 15.3 | 72.44 | 15.7 | 70.82 | 90.64 |
| 5 | 122 | 10.6 | 0.00 | 10.9 | 101.84 | 11.2 | 98.81 | 11.6 | 95.96 | 11.9 | 93.27 | 12.2 | 90.73 | 12.6 | 88.32 | 13.0 | 85.15 | 13.5 | 82.20 | 14.0 | 79.45 | 14.2 | 78.24 | 14.4 | 77.06 | 14.6 | 75.92 | 15.2 | 73.00 | 15.8 | 70.30 | 16.4 | 67.79 | 85.57 |
| 5 | 127 | 9.9 | 0.00 | 10.2 | 108.31 | 10.6 | 104.40 | 11.0 | 100.76 | 11.0 | 97.80 | 11.7 | 95.00 | 12.0 | 92.36 | 12.4 | 89.14 | 12.9 | 86.14 | 13.3 | 83.34 | 13.7 | 80.90 | 14.1 | 78.61 | 14.5 | 76.44 |  |  |  |  |  |  |  |
| 6 | 108 | 10.4 | 0.00 | 10.0 | 205.25 | 11.2 | 197.92 | 11.6 | 191.10 | 12.0 | 184.73 | 12.4 | 178.77 | 12.8 | 173.18 | 13.3 | 167.30 | 13.7 | 161.80 | 14.2 | 156.66 | 14.5 | 152.88 | 14.9 | 149.27 | 15.2 | 145.84 | 15.8 | 140.00 | 16.5 | 134.62 | 17.1 | 129.63 | 168.41 |
| 6 | 120 | 9.6 | 0.00 | 10.0 | 222.79 | 10.4 | 214.18 | 10.8 | 206.21 | 11.2 | 197.63 | 11.7 | 189.73 | 12.2 | 182.45 | 12.5 | 176.87 | 12.9 | 171.62 | 13.3 | 166.67 | 13.9 | 160.05 | 14.4 | 153.94 | 15.0 | 148.28 | 15.4 | 144.26 | 15.8 | 140.45 | 16.2 | 136.84 | 179.14 |
| 6 | 123 | 10.4 | 0.00 | 10.0 | 205.89 | 11.1 | 199.11 | 11.5 | 192.76 | 11.9 | 185.76 | 12.4 | 179.25 | 12.8 | 173.18 | 13.3 | 166.46 | 13.8 | 160.25 | 14.4 | 154.48 | 14.8 | 149.44 | 15.3 | 144.73 | 15.8 | 140.30 | 16.2 | 136.55 | 16.7 | 133.00 | 17.1 | 129.63 | 168.03 |
| 6 | 128 | 9.8 | 0.00 | 10.1 | 219.12 | 10.5 | 211.45 | 10.9 | 204.31 | 11.3 | 196.17 | 11.8 | 188.66 | 12.2 | 181.70 | 12.7 | 174.55 | 13.2 | 167.93 | 13.7 | 161.80 | 14.2 | 155.74 | 14.8 | 150.12 | 15.3 | 144.88 | 15.7 | 141.19 | 16.1 | 137.68 | 16.5 | 134.35 | 176.58 |
| 6 | 131 | 10.3 | 0.00 | 10.7 | 207.17 | 11.1 | 199.71 | 11.5 | 192.76 | 11.9 | 187.07 | 12.2 | 181.70 | 12.6 | 176.63 | 12.9 | 171.84 | 13.3 | 167.30 | 13.6 | 162.99 | 14.0 | 158.34 | 14.4 | 153.94 | 14.8 | 149.78 | 15.4 | 144.41 | 15.9 | 139.42 | 16.5 | 134.76 | 172.15 |
| 7 | 109 | 10.3 | 0.00 | 10.3 | 0.00 | 11.0 | 0.00 | 11.0 | 0.00 | 11.4 | 0.00 | 11.7 | 0.00 | 12.1 | 0.00 | 12.6 | 0.00 | 13.0 | 0.00 | 13.5 | 0.00 | 14.0 | 0.00 | 14.5 | 0.00 | 15.0 | 0.00 | 15.5 | 0.00 | 15.5 | 0.00 | 16.4 | 0.00 | 0.00 |
| 7 | 124 | 10.2 | 0.00 | 10.6 | 0.00 | 10.9 | 0.00 | 11.3 | 0.00 | 11.6 | 0.00 | 11.9 | 0.00 | 12.3 | 0.00 | 12.8 | 0.00 | 13.3 | 0.00 | 13.8 | 0.00 | 14.1 | 0.00 | 14.4 | 0.00 | 14.7 | 0.00 | 15.2 | 0.00 | 15.7 | 0.00 | 16.2 | 0.00 | 0.00 |
| 7 | 135 | 10.3 | 0.00 | 10.6 | 0.00 | 11.0 | 0.00 | 11.3 | 0.00 | 11.7 | 0.00 | 12.0 | 0.00 | 12.4 | 0.00 | 12.8 | 0.00 | 13.2 | 0.00 | 13.7 | 0.00 | 14.0 | 0.00 | 14.4 | 0.00 | 14.8 | 0.00 | 15.3 | 0.00 | 15.9 | 0.00 | 16.4 | 0.00 | 0.00 |

**Missed Doses:**
Day 2 - Pig 121 did not eat entire PM dose (ate approximately 90%).  Daily dose adjusted to 95%.

On day 11 (10/13/2006), pig 127 was noted during weighing to have a rectal prolapse and was euthanized.

**TABLE D-10  ANIMAL HEALTH**

**Naxcel Treatment**[a]

| First Day of Treatment | Pig | Group | Treatment Duration |
|---|---|---|---|
| Day 0 (10/2/06) | 101 | 1 | 3 Days |
| | 109 | 7 | 6 Days |
| Day 1 (10/3/06) | 118 | 5 | 3 Days |
| | 126 | 3 | |
| | 133 | 2 | |
| | 138 | 3 | |
| Day 3 (10/5/06) | 114 | 1 | 3 Days |
| | 125 | 3 | |
| Day 5 (10/7/06) | 124 | 7 | 3 Days |
| Day 6 (10/8/06) | 120 | 6 | 3 Days |
| | 105 | 2 | 3 Days[b] |
| Day 7 (10/9/06) | 110 | 1 | 3 Days |
| | 118 | 5 | |

[a] All treatments are 1mL/day of Naxcel
[b] Received two doses in the day (on in the morning, one in the evening

**Necropsy**
Pig 131 (group 6) was noted to have a small umbilical abcess.

**Other**
On day 11 (10/13/06), pig 127 (group 5) was noted during weighing to have a rectal prolapse and was euthanized.

Navy1_Appendix A.xls (A-4_Health)

**TABLE D-11  URINE VOLUMES**

| Group | Pig Number | Urine Collection[a] | | |
|---|---|---|---|---|
| | | **U-1**<br>Days 6-7 | **U-2**<br>Days 9-10 | **U-3**<br>Days 12-13 |
| 1 | 101 | 10720 | 9460 | 16980 |
| | 103 | 6280 | 15650 | 24520 |
| | 110 | 6152 | 8000 | 12800 |
| | 114 | 18049 | 16090 | 11200 |
| | 132 | 8400 | 5920 | 6730 |
| 2 | 105 | 2610 | 4575 | 5660 |
| | 107 | 4590 | 5190 | 6850 |
| | 121 | 9200 | 11050 | 10520 |
| | 130 | 17460 | 14950 | 22740 |
| | 133 | 11500 | 19780 | 17960 |
| 3 | 104 | 5270 | 8920 | 11880 |
| | 113 | 18107 | 12845 | 11860 |
| | 125 | 11000 | 9940 | 17530 |
| | 126 | 9480 | 7600 | 14300 |
| | 138 | 7125 | 5080 | 6080 |
| 4 | 102 | 12750 | 21860 | 23000 |
| | 116 | 26183 | 26330 | 19020 |
| | 129 | 13500 | 9930 | 17040 |
| | 134 | 16840 | 8116 | 8067 |
| | 137 | 7880 | 9220 | 11420 |
| 5 | 106 | 10137 | 12200 | 24230 |
| | 117 | 5080 | 3500 | 13240 |
| | 118 | 7270 | 9880 | 9000 |
| | 122 | 5990 | 5980 | 5050 |
| | 127 | 4600 | 3890 | no sample[b] |
| 6 | 108 | 7490 | 5660 | 8840 |
| | 120 | 5160 | 8660 | 5880 |
| | 123 | 5500 | 8520 | 13620 |
| | 128 | 10860 | 12920 | 19920 |
| | 131 | 11290 | 14520 | 36450 |
| 7 | 109 | 3900 | 4340 | 4510 |
| | 124 | 6940 | 13680 | 19960 |
| | 135 | 1340 | 2710 | 5640 |

Units = milliliters
[a] Urine was collected over 48-hour periods.
[a] Pig 127 was euthanized on day 11 due to a rectal prolapse.

Navy1_Appendix A.xls (A-5_UrineVol)

## TABLE D-12 URINARY ARSENIC ANALYTICAL RESULTS FOR STUDY SAMPLES

| Sample Number | Tag Number | Pig Number | Group | Material Administered | Urine Collection Days | 48-hr Dose (ug/48hr) | 48-hr BWAdj Dose (ug/kg-48hr) | Q | Reported Conc (ng/mL) | AdjConc*( ng/mL) | Urine Volume (mL) | Total Excreted (ug/48hrs) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Navy1As-114-U1 | Navy1As-109 | 114 | 1 | Sodium Arsenate | 6/7 | 692.73 | 56.33 | | 38 | 38 | 18049 | 686 |
| Navy1As-132-U1 | Navy1As-133 | 132 | 1 | Sodium Arsenate | 6/7 | 692.73 | 52.99 | | 76 | 76 | 8400 | 638 |
| Navy1As-101-U1 | Navy1As-106 | 101 | 1 | Sodium Arsenate | 6/7 | 692.73 | 55.21 | | 56 | 56 | 10720 | 600 |
| Navy1As-110-U1 | Navy1As-125 | 110 | 1 | Sodium Arsenate | 6/7 | 692.73 | 53.51 | | 98 | 98 | 6152 | 603 |
| Navy1As-103-U1 | Navy1As-141 | 103 | 1 | Sodium Arsenate | 6/7 | 692.73 | 52.7 | | 119 | 119 | 6280 | 747 |
| Navy1As-105-U1 | Navy1As-138 | 105 | 2 | Sodium Arsenate | 6/7 | 1385.45 | 114.28 | | 380 | 380 | 2610 | 992 |
| Navy1As-107-U1 | Navy1As-114 | 107 | 2 | Sodium Arsenate | 6/7 | 1385.45 | 110.65 | | 250 | 250 | 4590 | 1148 |
| Navy1As-121-U1 | Navy1As-108 | 121 | 2 | Sodium Arsenate | 6/7 | 1385.45 | 110.85 | | 141 | 141 | 9200 | 1297 |
| Navy1As-130-U1 | Navy1As-102 | 130 | 2 | Sodium Arsenate | 6/7 | 1385.45 | 108.27 | | 68 | 68 | 17460 | 1187 |
| Navy1As-133-U1 | Navy1As-137 | 133 | 2 | Sodium Arsenate | 6/7 | 1385.45 | 106.19 | | 107 | 107 | 11500 | 1231 |
| Navy1As-104-U1 | Navy1As-118 | 104 | 3 | Sodium Arsenate | 6/7 | 2770.91 | 203.4 | | 410 | 410 | 5270 | 2161 |
| Navy1As-113-U1 | Navy1As-105 | 113 | 3 | Sodium Arsenate | 6/7 | 2770.91 | 204.91 | | 144 | 144 | 18107 | 2607 |
| Navy1As-125-U1 | Navy1As-131 | 125 | 3 | Sodium Arsenate | 6/7 | 2770.91 | 223.52 | | 220 | 220 | 11000 | 2420 |
| Navy1As-126-U1 | Navy1As-113 | 126 | 3 | Sodium Arsenate | 6/7 | 2770.91 | 215.72 | | 240 | 240 | 9480 | 2275 |
| Navy1As-138-U1 | Navy1As-104 | 138 | 3 | Sodium Arsenate | 6/7 | 2770.91 | 228.14 | | 320 | 320 | 7125 | 2280 |
| Navy1As-134-U1 | Navy1As-121 | 134 | 4 | Test Material 1 | 6/7 | 1108.36 | 89.59 | | 14 | 14 | 16840 | 236 |
| Navy1As-137-U1 | Navy1As-128 | 137 | 4 | Test Material 1 | 6/7 | 1108.36 | 89.24 | | 3.7 | 3.7 | 7880 | 29 |
| Navy1As-116-U1 | Navy1As-120 | 116 | 4 | Test Material 1 | 6/7 | 1108.36 | 91.25 | | 6.4 | 6.4 | 26183 | 168 |
| Navy1As-102-U1 | Navy1As-130 | 102 | 4 | Test Material 1 | 6/7 | 1108.36 | 81.51 | | 9.3 | 9.3 | 12750 | 119 |
| Navy1As-129-U1 | Navy1As-139 | 129 | 4 | Test Material 1 | 6/7 | 1108.36 | 89.6 | | 11 | 11 | 13500 | 149 |
| Navy1As-127-U1 | Navy1As-112 | 127 | 5 | Test Material 1 | 6/7 | 2216.73 | 175.29 | | 71 | 71 | 4600 | 327 |
| Navy1As-122-U1 | Navy1As-126 | 122 | 5 | Test Material 1 | 6/7 | 2216.73 | 167.35 | | 41 | 41 | 5990 | 246 |
| Navy1As-118-U1 | Navy1As-103 | 118 | 5 | Test Material 1 | 6/7 | 2216.73 | 176.69 | | 29 | 29 | 7270 | 211 |
| Navy1As-117-U1 | Navy1As-124 | 117 | 5 | Test Material 1 | 6/7 | 2216.73 | 168.29 | | 44 | 44 | 5080 | 224 |
| Navy1As-106-U1 | Navy1As-135 | 106 | 5 | Test Material 1 | 6/7 | 2216.73 | 174.24 | | 31 | 31 | 10137 | 314 |
| Navy1As-131-U1 | Navy1As-134 | 131 | 6 | Test Material 1 | 6/7 | 4433.45 | 339.14 | | 53 | 53 | 11290 | 598 |
| Navy1As-128-U1 | Navy1As-115 | 128 | 6 | Test Material 1 | 6/7 | 4433.45 | 342.48 | | 48 | 48 | 10860 | 521 |
| Navy1As-123-U1 | Navy1As-107 | 123 | 6 | Test Material 1 | 6/7 | 4433.45 | 326.71 | | 97 | 97 | 5500 | 534 |
| Navy1As-120-U1 | Navy1As-101 | 120 | 6 | Test Material 1 | 6/7 | 4433.45 | 348.48 | | 84 | 84 | 5160 | 433 |
| Navy1As-108-U1 | Navy1As-122 | 108 | 6 | Test Material 1 | 6/7 | 4433.45 | 329.11 | | 82 | 82 | 7490 | 614 |
| Navy1As-135-U1 | Navy1As-111 | 135 | 7 | Control | 6/7 | 0 | 0 | | 7.6 | 7.6 | 1340 | 10 |
| Navy1As-124-U1 | Navy1As-132 | 124 | 7 | Control | 6/7 | 0 | 0 | | 5.9 | 5.9 | 6940 | 41 |
| Navy1As-109-U1 | Navy1As-136 | 109 | 7 | Control | 6/7 | 0 | 0 | | 2 | 2 | 3900 | 8 |
| Navy1As-101-U2 | Navy1As-147 | 101 | 1 | Sodium Arsenate | 9/10 | 692.73 | 50.03 | | 73 | 73 | 9460 | 691 |
| Navy1As-103-U2 | Navy1As-170 | 103 | 1 | Sodium Arsenate | 9/10 | 692.73 | 47.54 | | 43 | 43 | 15650 | 673 |
| Navy1As-110-U2 | Navy1As-160 | 110 | 1 | Sodium Arsenate | 9/10 | 692.73 | 48.37 | | 71 | 71 | 8000 | 568 |
| Navy1As-114-U2 | Navy1As-146 | 114 | 1 | Sodium Arsenate | 9/10 | 692.73 | 51.42 | | 41 | 41 | 16090 | 660 |
| Navy1As-132-U2 | Navy1As-150 | 132 | 1 | Sodium Arsenate | 9/10 | 692.73 | 48.37 | | 114 | 114 | 5920 | 675 |
| Navy1As-121-U2 | Navy1As-151 | 121 | 2 | Sodium Arsenate | 9/10 | 1385.45 | 102.09 | | 115 | 115 | 11050 | 1271 |
| Navy1As-130-U2 | Navy1As-167 | 130 | 2 | Sodium Arsenate | 9/10 | 1385.45 | 97.43 | | 83 | 83 | 14950 | 1241 |
| Navy1As-107-U2 | Navy1As-161 | 107 | 2 | Sodium Arsenate | 9/10 | 1385.45 | 99.53 | | 190 | 190 | 5190 | 986 |
| Navy1As-105-U2 | Navy1As-163 | 105 | 2 | Sodium Arsenate | 9/10 | 1385.45 | 104.01 | | 290 | 290 | 4575 | 1327 |
| Navy1As-133-U2 | Navy1As-155 | 133 | 2 | Sodium Arsenate | 9/10 | 1385.45 | 97.41 | | 71 | 71 | 19780 | 1404 |
| Navy1As-104-U2 | Navy1As-177 | 104 | 3 | Sodium Arsenate | 9/10 | 2770.91 | 187.89 | | 290 | 290 | 8920 | 2587 |
| Navy1As-113-U2 | Navy1As-164 | 113 | 3 | Sodium Arsenate | 9/10 | 2770.91 | 188.85 | | 230 | 230 | 12845 | 2954 |
| Navy1As-125-U2 | Navy1As-142 | 125 | 3 | Sodium Arsenate | 9/10 | 2770.91 | 203.42 | | 240 | 240 | 9940 | 2386 |
| Navy1As-126-U2 | Navy1As-152 | 126 | 3 | Sodium Arsenate | 9/10 | 2770.91 | 195.86 | | 330 | 330 | 7600 | 2508 |
| Navy1As-138-U2 | Navy1As-156 | 138 | 3 | Sodium Arsenate | 9/10 | 2770.91 | 204.56 | | 500 | 500 | 5080 | 2540 |
| Navy1As-102-U2 | Navy1As-180 | 102 | 4 | Test Material 1 | 9/10 | 1108.36 | 74.41 | | 9 | 9 | 21860 | 197 |
| Navy1As-137-U2 | Navy1As-145 | 137 | 4 | Test Material 1 | 9/10 | 1108.36 | 80.19 | | 19 | 19 | 9220 | 175 |
| Navy1As-116-U2 | Navy1As-171 | 116 | 4 | Test Material 1 | 9/10 | 1108.36 | 83.04 | | 6.6 | 6.6 | 26330 | 174 |
| Navy1As-134-U2 | Navy1As-172 | 134 | 4 | Test Material 1 | 9/10 | 1108.36 | 80.35 | | 21 | 21 | 8116 | 170 |
| Navy1As-129-U2 | Navy1As-166 | 129 | 4 | Test Material 1 | 9/10 | 1108.36 | 81.07 | | 15 | 15 | 9930 | 149 |
| Navy1As-106-U2 | Navy1As-178 | 106 | 5 | Test Material 1 | 9/10 | 2216.73 | 161.24 | | 31 | 31 | 12200 | 378 |
| Navy1As-117-U2 | Navy1As-157 | 117 | 5 | Test Material 1 | 9/10 | 2216.73 | 149.85 | | 70 | 70 | 3500 | 245 |
| Navy1As-118-U2 | Navy1As-173 | 118 | 5 | Test Material 1 | 9/10 | 2216.73 | 159.23 | | 21 | 21 | 9880 | 207 |
| Navy1As-122-U2 | Navy1As-175 | 122 | 5 | Test Material 1 | 9/10 | 2216.73 | 155.3 | | 33 | 33 | 5980 | 197 |
| Navy1As-127-U2 | Navy1As-168 | 127 | 5 | Test Material 1 | 9/10 | 2216.73 | 159.51 | | 70 | 70 | 3890 | 272 |
| Navy1As-108-U2 | Navy1As-153 | 108 | 6 | Test Material 1 | 9/10 | 4433.45 | 302.15 | | 81 | 81 | 5660 | 458 |
| Navy1As-131-U2 | Navy1As-162 | 131 | 6 | Test Material 1 | 9/10 | 4433.45 | 312.28 | | 47 | 47 | 14520 | 682 |
| Navy1As-128-U2 | Navy1As-148 | 128 | 6 | Test Material 1 | 9/10 | 4433.45 | 305.86 | | 43 | 43 | 12920 | 556 |
| Navy1As-120-U2 | Navy1As-169 | 120 | 6 | Test Material 1 | 9/10 | 4433.45 | 313.99 | | 59 | 59 | 8660 | 511 |
| Navy1As-123-U2 | Navy1As-165 | 123 | 6 | Test Material 1 | 9/10 | 4433.45 | 294.17 | | 62 | 62 | 8520 | 528 |
| Navy1As-109-U2 | Navy1As-174 | 109 | 7 | Control | 9/10 | 0 | 0 | | 5.6 | 5.6 | 4340 | 24 |
| Navy1As-124-U2 | Navy1As-159 | 124 | 7 | Control | 9/10 | 0 | 0 | | 2 | 2 | 13680 | 27 |
| Navy1As-135-U2 | Navy1As-143 | 135 | 7 | Control | 9/10 | 0 | 0 | | 4.6 | 4.6 | 2710 | 12 |
| ENSR1-445-U2 | ENSR1-183 | 445 | 6 | Test Material 2 | 9/10 | 3495.42 | 224.11 | | 70 | 70 | 20740 | 1452 |

| Sample Number | Tag Number | Pig Number | Group | Material Administered | Urine Collection Days | 48-hr Dose (ug/48hr) | 48-hr BWAdj Dose (ug/kg-48hr) | Q | Reported Conc (ng/mL) | AdjConc* (ng/mL) | Urine Volume (mL) | Total Excreted (ug/48hrs) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ENSR1-452-U2 | ENSR1-165 | 452 | 7 | Control | 9/10 | 0 | 0 | < | 1 | 0.5 | 23980 | 12 |
| ENSR1-461-U2 | ENSR1-144 | 461 | 7 | Control | 9/10 | 0 | 0 | | 1 | 1 | 7600 | 8 |
| ENSR1-464-U2 | ENSR1-174 | 464 | 7 | Control | 9/10 | 0 | 0 | | 2 | 2 | 5140 | 10 |
| ENSR1-467-U2 | ENSR1-147 | 467 | 7 | Control | 9/10 | 0 | 0 | < | 1 | 0.5 | 7160 | 4 |
| ENSR1-432-U2 | ENSR1-180 | 432 | 7 | Control | 9/10 | 0 | 0 | < | 1 | 0.5 | 14560 | 7 |
| Navy1As-101-U3 | Navy1As-188 | 101 | 1 | Sodium Arsenate | 12/13 | 692.73 | 45.51 | | 41 | 41 | 16980 | 696 |
| Navy1As-132-U3 | Navy1As-215 | 132 | 1 | Sodium Arsenate | 12/13 | 692.73 | 44.13 | | 99 | 99 | 6730 | 666 |
| Navy1As-114-U3 | Navy1As-184 | 114 | 1 | Sodium Arsenate | 12/13 | 692.73 | 46.82 | | 51 | 51 | 11200 | 571 |
| Navy1As-110-U3 | Navy1As-192 | 110 | 1 | Sodium Arsenate | 12/13 | 692.73 | 44.06 | | 49 | 49 | 12800 | 627 |
| Navy1As-103-U3 | Navy1As-195 | 103 | 1 | Sodium Arsenate | 12/13 | 692.73 | 43.51 | | 27 | 27 | 24520 | 662 |
| Navy1As-133-U3 | Navy1As-222 | 133 | 2 | Sodium Arsenate | 12/13 | 1385.45 | 89.41 | | 74 | 74 | 17960 | 1329 |
| Navy1As-130-U3 | Navy1As-216 | 130 | 2 | Sodium Arsenate | 12/13 | 1385.45 | 87.85 | | 64 | 64 | 22740 | 1455 |
| Navy1As-121-U3 | Navy1As-221 | 121 | 2 | Sodium Arsenate | 12/13 | 1385.45 | 93.16 | | 110 | 110 | 10520 | 1157 |
| Navy1As-107-U3 | Navy1As-205 | 107 | 2 | Sodium Arsenate | 12/13 | 1385.45 | 91.16 | | 190 | 190 | 6850 | 1302 |
| Navy1As-105-U3 | Navy1As-218 | 105 | 2 | Sodium Arsenate | 12/13 | 1385.45 | 94.27 | | 240 | 240 | 5660 | 1358 |
| Navy1As-113-U3 | Navy1As-193 | 113 | 3 | Sodium Arsenate | 12/13 | 2770.91 | 172.42 | | 220 | 220 | 11860 | 2609 |
| Navy1As-138-U3 | Navy1As-190 | 138 | 3 | Sodium Arsenate | 12/13 | 2770.91 | 183.87 | | 340 | 340 | 6080 | 2067 |
| Navy1As-125-U3 | Navy1As-200 | 125 | 3 | Sodium Arsenate | 12/13 | 2770.91 | 185.39 | | 160 | 160 | 17530 | 2805 |
| Navy1As-104-U3 | Navy1As-220 | 104 | 3 | Sodium Arsenate | 12/13 | 2770.91 | 173.48 | | 230 | 230 | 11880 | 2732 |
| Navy1As-126-U3 | Navy1As-194 | 126 | 3 | Sodium Arsenate | 12/13 | 2770.91 | 180.55 | | 190 | 190 | 14300 | 2717 |
| Navy1As-134-U3 | Navy1As-187 | 134 | 4 | Test Material 1 | 12/13 | 1108.36 | 71.99 | | 20 | 20 | 8067 | 161 |
| Navy1As-137-U3 | Navy1As-210 | 137 | 4 | Test Material 1 | 12/13 | 1108.36 | 72.82 | | 14 | 14 | 11420 | 160 |
| Navy1As-129-U3 | Navy1As-202 | 129 | 4 | Test Material 1 | 12/13 | 1108.36 | 73.67 | | 9 | 9 | 17040 | 153 |
| Navy1As-116-U3 | Navy1As-219 | 116 | 4 | Test Material 1 | 12/13 | 1108.36 | 75.8 | | 13 | 13 | 19020 | 247 |
| Navy1As-102-U3 | Navy1As-209 | 102 | 4 | Test Material 1 | 12/13 | 1108.36 | 68.22 | | 7.2 | 7.2 | 23000 | 166 |
| Navy1As-117-U3 | Navy1As-206 | 117 | 5 | Test Material 1 | 12/13 | 2216.73 | 134.78 | | 28 | 28 | 13240 | 371 |
| Navy1As-118-U3 | Navy1As-214 | 118 | 5 | Test Material 1 | 12/13 | 2216.73 | 146.58 | | 25 | 25 | 9000 | 225 |
| Navy1As-122-U3 | Navy1As-204 | 122 | 5 | Test Material 1 | 12/13 | 2216.73 | 143.3 | | 59 | 59 | 5050 | 298 |
| Navy1As-127-U3 | Navy1As-199 | 127 | 5 | Test Material 1 | 12/13 | | | | | | | |
| Navy1As-106-U3 | Navy1As-201 | 106 | 5 | Test Material 1 | 12/13 | 2216.73 | 149.56 | | 15 | 15 | 24230 | 363 |
| Navy1As-131-U3 | Navy1As-186 | 131 | 6 | Test Material 1 | 12/13 | 4433.45 | 283.83 | | 19 | 19 | 36450 | 693 |
| Navy1As-123-U3 | Navy1As-198 | 123 | 6 | Test Material 1 | 12/13 | 4433.45 | 269.56 | | 37 | 37 | 13620 | 504 |
| Navy1As-120-U3 | Navy1As-196 | 120 | 6 | Test Material 1 | 12/13 | 4433.45 | 284.7 | | 83 | 83 | 5880 | 488 |
| Navy1As-128-U3 | Navy1As-217 | 128 | 6 | Test Material 1 | 12/13 | 4433.45 | 278.88 | | 26 | 26 | 19920 | 518 |
| Navy1As-108-U3 | Navy1As-185 | 108 | 6 | Test Material 1 | 12/13 | 4433.45 | 274.62 | | 66 | 66 | 8840 | 583 |
| Navy1As-109-U3 | Navy1As-189 | 109 | 7 | Control | 12/13 | 0 | 0 | | 2 | 2 | 4510 | 9 |
| Navy1As-124-U3 | Navy1As-203 | 124 | 7 | Control | 12/13 | 0 | 0 | < | 1 | 0.5 | 19960 | 10 |
| Navy1As-135-U3 | Navy1As-207 | 135 | 7 | Control | 12/13 | 0 | 0 | | 2 | 2 | 5640 | 11 |

Q = Data qualifier
*Non-detects taken at one-half the detection limit.

# TABLE D-13  ARSENIC ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

**Blind Duplicates**

| Tag Number | Group | Event | DL | Duplicate Conc | Original Pig # |
|---|---|---|---|---|---|
| Navy1As-110 | 7 | U1 | 1 | 7.6 | 135 |
| Navy1As-127 | 2 | U1 | 1 | 105 | 133 |
| Navy1As-117 | 6 | U1 | 1 | 89 | 120 |
| Navy1As-179 | 1 | U2 | 1 | 74 | 101 |
| Navy1As-154 | 3 | U2 | 10 | 470 | 138 |
| Navy1As-149 | 5 | U2 | 1 | 37 | 122 |
| Navy1As-183 | 2 | U3 | 5 | 200 | 107 |
| Navy1As-211 | 4 | U3 | 1 | 6.4 | 116 |
| Navy1As-197 | 6 | U3 | 1 | 16 | 131 |

**PE Sample Analysis**

| Tag Number | QC Sample | Nominal Conc | Measured Conc | DL |
|---|---|---|---|---|
| Navy1As-212 | Control Urine | 0 | 2 | 1 |
| Navy1As-116 | Control Urine | 0 | 3 | 1 |
| Navy1As-158 | Sodium arsenate | 20 | 21 | 1 |
| Navy1As-123 | Sodium arsenate | 100 | 104 | 1 |
| Navy1As-191 | Sodium arsenate | 400 | 400 | 10 |
| Navy1As-208 | Sodium arsenite | 20 | 21 | 1 |
| Navy1As-176 | Sodium arsenite | 100 | 99 | 1 |
| Navy1As-129 | Sodium arsenite | 400 | 370 | 10 |
| Navy1As-119 | Dimethyl arsenic acid | 20 | 24 | 1 |
| Navy1As-213 | Dimethyl arsenic acid | 100 | 104 | 1 |
| Navy1As-181 | Dimethyl arsenic acid | 400 | 390 | 10 |
| Navy1As-140 | Disodium methylarsenate | 20 | 21 | 1 |
| Navy1As-144 | Disodium methylarsenate | 100 | 106 | 1 |
| Navy1As-182 | Disodium methylarsenate | 400 | 390 | 10 |

**Laboratory Control Standards**

| Reference Material | Certified Mean | DL | Measured Conc |
|---|---|---|---|
| NIST 2670a-L | (3) | 3 | 5 |
| NIST 2670a-H | 220 ± 10 | 5 | 220 |
| NIST 2670a-H | 220 ± 10 | 5 | 210 |
| NIST 2670a-L | (3) | 3 | <3 |
| NIST 2670a-H | 220 ± 10 | 5 | 220 |
| NIST 2670a-H | 220 ± 10 | 5 | 210 |
| NIST 1640 | 0.0267 +/-0.0004 | 0.001 | 0.029 |
| NRCC TORT-2 | 21.6 +/-1.8 | 0.2 | 22.3 |

**Blanks**

| Tag Number | DL | Measured Conc |
|---|---|---|
| Blank-1 | 1 | <1 |
| Blank-2 | 1 | <1 |
| Blank-3 | 1 | <1 |
| Blank-4 | 1 | <1 |
| Blank-5 | 1 | <1 |
| Blank-6 | 1 | <1 |

**Duplicates**

| Tag Number | DL | Duplicate Result | Original Sample Conc |
|---|---|---|---|
| Navy1-103 | 1 | 30 | 29 |
| Navy1-113 | 5 | 240 | 240 |
| Navy1-123 | 1 | 106 | 104 |
| Navy1-135 | 1 | 31 | 31 |
| Navy1-142 | 5 | 270 | 240 |
| Navy1-152 | 10 | 340 | 330 |
| Navy1-163 | 10 | 300 | 290 |
| Navy1-173 | 1 | 22 | 21 |
| Navy1-182 | 10 | 400 | 390 |
| Navy1-192 | 1 | 49 | 49 |
| Navy1-204 | 1 | 63 | 59 |
| Navy1-212 | 1 | 2 | 2 |
| Navy1-221 | 1 | 109 | 110 |

**Spikes (nominal spike: 200 ng/mL)**

| Tag Number | DL | Original Sample Conc | Spiked Result |
|---|---|---|---|
| Navy1-108 | 1 | 141 | 360 |
| Navy1-116 | 1 | 3 | 210 |
| Navy1-128 | 1 | 19 | 210 |
| Navy1-139 | 1 | 11 | 210 |
| Navy1-147 | 1 | 73 | 280 |
| Navy1-157 | 1 | 70 | 280 |
| Navy1-168 | 1 | 70 | 280 |
| Navy1-177 | 10 | 290 | 520* |
| Navy1-187 | 1 | 20 | 230 |
| Navy1-198 | 1 | 37 | 240 |
| Navy1-208 | 1 | 21 | 230 |
| Navy1-216 | 1 | 64 | 250 |

DL = Detection limit
Units:  ng/mL (urine), ug/mL (water), ug/g (tissue)
*Nominal spike amount too low (less than original sample concentration)

**Relative bioavailable Cr of soil from the McClellan Air Force Base**

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of chromium from a soil sample taken in the vicinity of McClellan Air Force Base The soil sample contained a chromium concentration of 593 ug/g. The relative bioavailability of chromium was assessed by comparing the absorption of chromium from the test material to that of a reference material (chromium chloride). Groups of five swine were given oral doses of chromium chloride or the test materials twice a day for 14 days; a group of three non-treated swine served as a control.  The amount of chromium absorbed by each animal was evaluated by measuring the amount of chromium excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12).  The urinary excretion fraction (UEF) (the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours) was calculated for both the test soil and chromium chloride using linear regression analysis. The relative bioavailability (RBA) of chromium in the test soil compared to that in chromium chloride was calculated as follows:

$$RBA = \frac{UEF\ (test\ material)}{UEF\ (chromium\ chloride)}$$

Results are summarized in Table D-3. Support detailed information of experimental data used to derive the RBA Cr for the McClellan soil follows Table D-3. The chromium RBA estimates are approximately 107% for the test material.   This indicates that the chromium in this material is as well absorbed as soluble chromium.  This relative bioavailability estimate may be used to improve accuracy and decrease uncertainty in estimating human health risks from exposure to this site-specific test material.  It is not clear why the estimate based on the day 6/7 urine collection has such wide variation.  A plausible explanation might be that fecal contamination of the urine sample resulting in falsely elevated levels.

**Table D-13.** Relative Bioavailable Cr of the McClellan Soil

## Navy2 Chromium - McClellan Air Force Base

| Measurement Endpoint | Estimated RBA (90% Confidence Interval) |
|---|---|
| Days 6/7 | 1.27 (0.60 - -16.91) |
| Days 9/10 | 0.75 (0.44 - 1.18) |
| Days 12/13 | 0.77 (0.16 - 2.06) |
| All Days | 1.07 (0.76 - 1.69) |

## TABLE D-14  DOSING PROTOCOL

| Group | Number of Animals | Dose Material Administered | Chromium Dose (µg/kg-day) | |
| --- | --- | --- | --- | --- |
| | | | Target | Actual [a] |
| 1 | 5 | Chromium Chloride | 250 | 306.9 |
| 2 | 5 | Chromium Chloride | 500 | 593.7 |
| 3 | 5 | Chromium Chloride | 750 | 866.7 |
| 4 | 5 | Test Material 2 | 400 | 426.7 |
| 5 | 5 | Test Material 2 | 650 | 729.4 |
| 6 | 5 | Test Material 2 | 900 | 999.7 |
| 7 | 3 | Control | 0 | 0.0 |

[a] Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0-14 for each animal and each group.

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day.  Doses were held constant based on a body weight of 13.0 kg, the expected mean weight during the exposure interval (14 days).  Actual mean body weight across all animals during the exposure interval was 11.7 kg.

# TABLE D-15  TYPICAL FEED COMPOSITION

**TestDiet 5TXP:  Porcine Grower Purified Diet with Low Lead**[1]

## INGREDIENTS

| | | | |
|---|---|---|---|
| Corn Starch, % | 25.2 | Potassium Phosphate, % | 0.87 |
| Sucrose, % | 20.9648 | Calcium Carbonate, % | 0.7487 |
| Glucose, % | 16 | Salt, % | 0.501 |
| Soy Protein Isolate, % | 14.9899 | Magnesium Sulfate, % | 0.1245 |
| Casein - Vitamin Free, % | 8.5 | DL-Methionine, % | 0.0762 |
| Powdered Cellulose, % | 6.7208 | Choline Chloride, % | 0.0586 |
| Corn Oil, % | 3.4046 | Vitamin/Mineral Premix, % | 0.0577 |
| Dicalcium Phosphate, % | 1.7399 | Sodium Selenite, % | 0.0433 |

## NUTRITIONAL PROFILE[2]

| **Protein, %** | **21** | **Fat, %** | | **3.5** |
|---|---|---|---|---|
| Arginine, % | 1.42 | Cholesterol, ppm | | 0 |
| Histidine, % | 0.61 | Linoleic Acid, % | | 1.95 |
| Isoleucine, % | 1.14 | Linolenic Acid, % | | 0.03 |
| Leucine, % | 1.95 | Arachidonic Acid, % | | 0 |
| Lysine, % | 1.56 | Omega-3 Fatty Acids, % | | 0.03 |
| Methionine, % | 0.49 | Total Saturated Fatty Acids, % | | 0.43 |
| Cystine, % | 0.23 | Total Monounsaturated Fatty Acids, % | | 0.82 |
| Phenylalanine, % | 1.22 | Polyunsaturated Fatty Acids, % | | 1.98 |
| Tyrosine, % | 1.03 | | | |
| Threonine, % | 0.88 | | | |
| Tryptophan, % | 0.32 | **Fiber (max), %** | | **6.8** |
| Valine, % | 1.16 | | | |
| Alanine, % | 0.95 | **Carbohydrates, %** | | **62.2** |
| Aspartic Acid, % | 2.33 | | | |
| Glutamic Acid, % | 4.96 | **Energy (kcal/g)** [3] | | **3.62** |
| Glycine, % | 0.79 | *From:* | *kcal* | *%* |
| Proline, % | 1.83 | Protein | 0.84 | 23.1 |
| Serine, % | 1.25 | Fat (ether extract) | 0.315 | 8.7 |
| Taurine, % | 0 | Carbohydrates | 2.487 | 68.3 |

| **Minerals** | | **Vitamins** | |
|---|---|---|---|
| Calcium, % | 0.8 | Vitamin A, IU/g | 1.7 |
| Phosphorus, % | 0.72 | Vitamin 0-3 (added), IU/g | 0.2 |
| Phosphorus (available), % | 0.4 | Vitamin E, IU/kg | 11 |
| Potassium, % | 0.27 | Vitamin K (as menadione), ppm | 0.52 |
| Magnesium, % | 0.04 | Thiamin Hydrochloride, ppm | 1 |
| Sodium, % | 0.3 | Ribonavin, ppm | 3.1 |
| Chlorine, % | 0.31 | Niacin, ppm | 13 |
| Fluorine, ppm | 0 | Pantothenic Acid, ppm | 9 |
| Iron, ppm | 82 | Folic Acid, ppm | 0.3 |
| Zinc, ppm | 84 | Pyridoxine, ppm | 1.7 |
| Manganese, ppm | 3 | Biotin, ppm | 0.1 |
| Copper, ppm | 4.9 | Vitamin B-12, mcg/kg | 15 |
| Cobalt, ppm | 0.1 | Choline Chloride, ppm | 410 |
| Iodine, ppm | 0.15 | Ascorbic Acid, ppm | 0 |
| Chromium, ppm | 0 | | |
| Molybdenum, ppm | 0.01 | | |
| Selenium, ppm | 0.26 | | |

## FOOTNOTES

[1] This special purified diet was originally developed for lead RBA studies.

[2] Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly. Nutrients expressed as percent of ration on an As Fed basis except where otherwise indicated.

[3] Energy (kcal/gm) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9,4 kcal/gm respectively.

Table 2-2_Feed.xls (PURINA)

**FIGURE D-15  BODY WEIGHT GAIN**

Mean Body Weight (kg) vs Day of Study

Legend: Group 1, Group 2, Group 3, Group 4, Group 5, Group 6, Group 7

1_Navy2 Weights.xls (Fig2-1_group weights)

**FIGURE D-16  URINARY CHROMIUM BLIND DUPLICATES**

Line of Equality

Primary Analysis (ng/mL)

Duplicate Analysis (ng/mL)

4_Navy2 Lab QC analysis.xls (Fig2-2_BlindDupes)
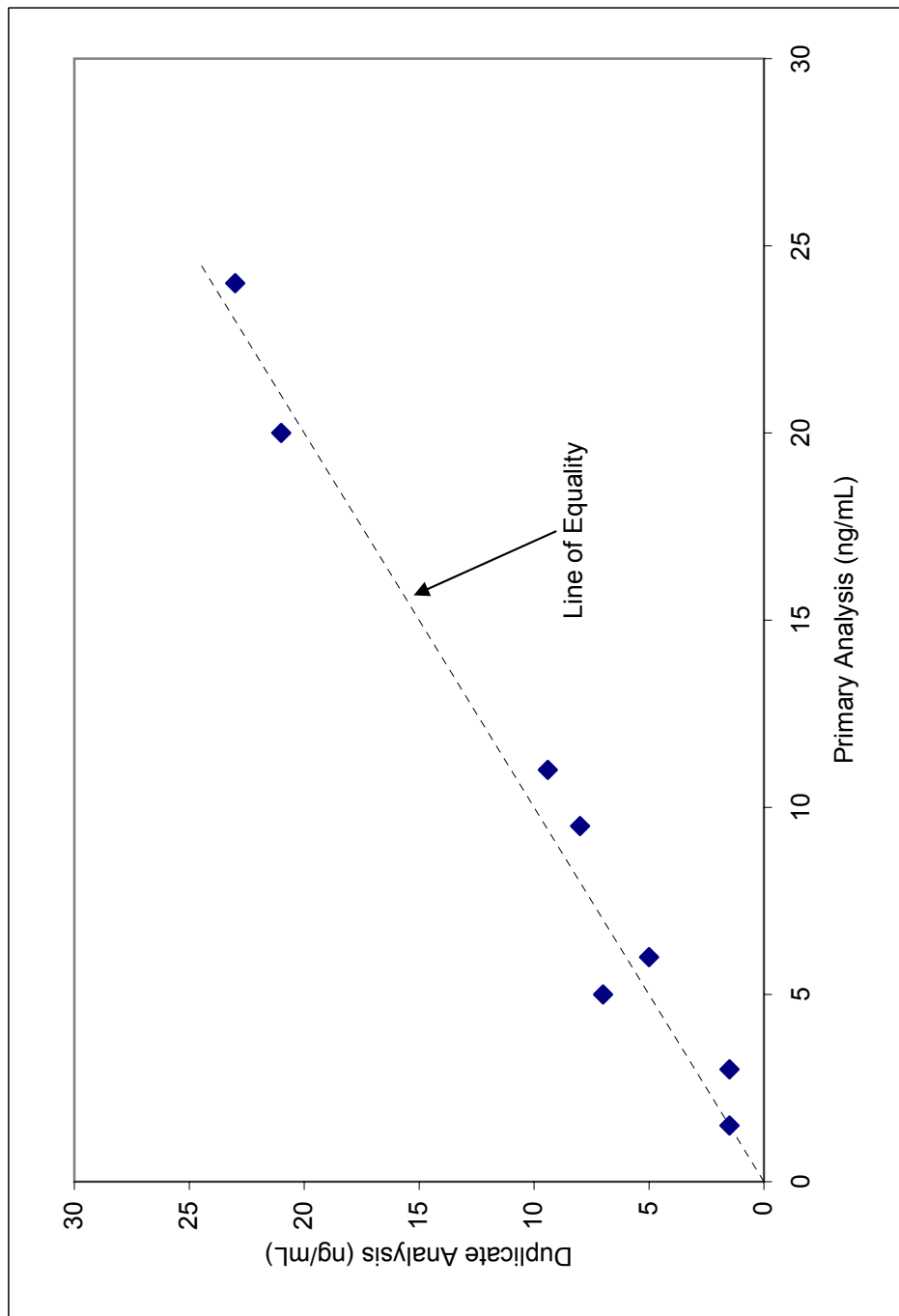
# FIGURE D-17  PERFORMANCE EVALUATION SAMPLES

**Chromium (III) Chloride**

4_Navy2 Lab QC analysis.xls (Fig2-3_PE)T
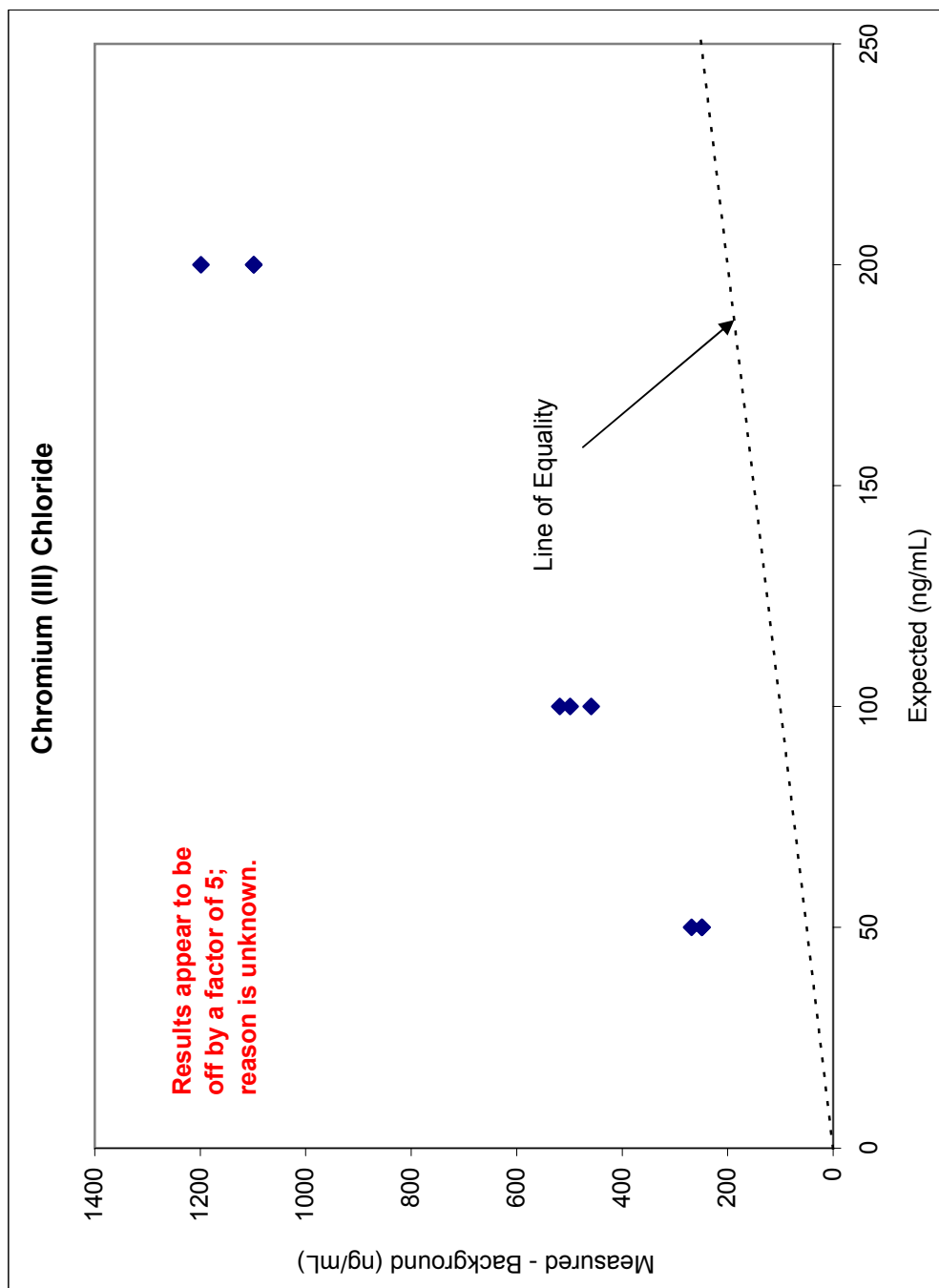
**Figure D-18.  Conceptual Model for Chromium Toxicokinetics**
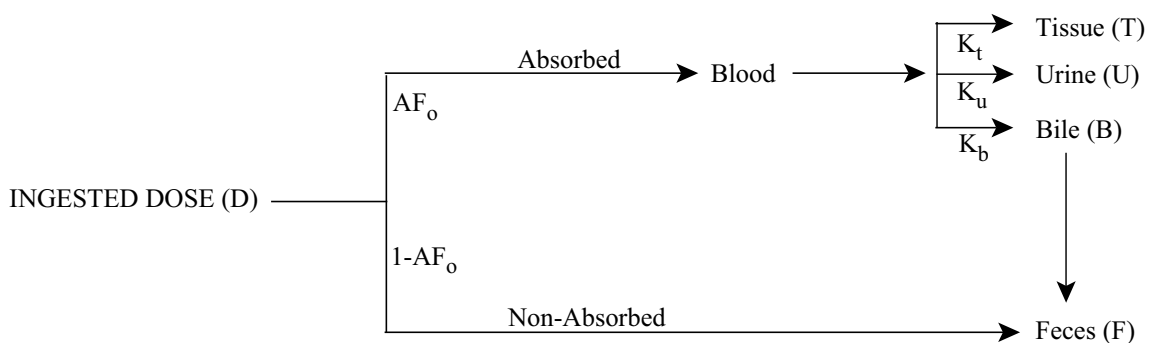


where:

$D$ = Ingested dose (ug)

$AF_o$ = Oral Absorption Fraction

$K_t$ = Fraction of absorbed chromium which is retained in tissues

$K_u$ = Fraction of absorbed chromium which is excreted in urine

$K_b$ = Fraction of absorbed chromium which is excreted in the bile

BASIC EQUATIONS:

Amount Absorbed (ug) $= D \times AF_o$

Amount Excreted (ug) $= \text{Amount absorbed} \times K_u$

$= D \times AF_o \times K_u$

Urinary Excretion Fraction (UEF) $= \text{Amount excreted / Amount Ingested}$

$= (D \times AF_o \times K_u) / D$

$= AF_o \times K_u$

Relative Bioavailability (x vs. y) $= UEF(x) / UEF(y)$

$= (AF_o(x) \times K_u) / (AF_o(y) \times K_u)$

$= AF_o(x) / AF_o(y)$

Fig 3-1_Toxicokinetics.wpd

# FIGURE D-19  URINARY EXCRETION OF CHROMIUM:  Days 6/7 (All Data)

## Reference Material (Chromium Chloride)
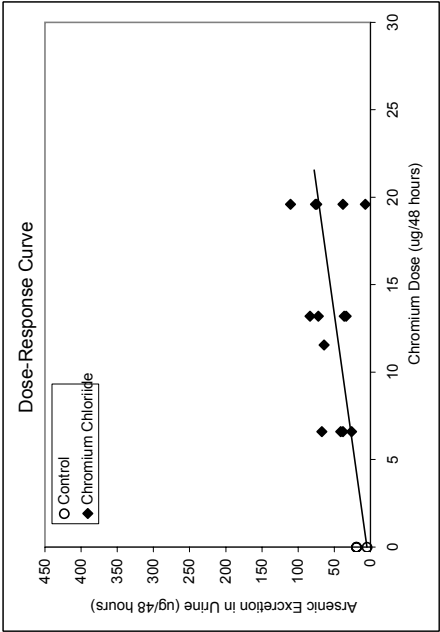
## Test Material 2 (McClellan Air Force Base)



Dose-Response Curve (Reference Material)



Residual Plot (Reference Material)



Dose-Response Curve (Test Material 2)



Residual Plot (Test Material 2)
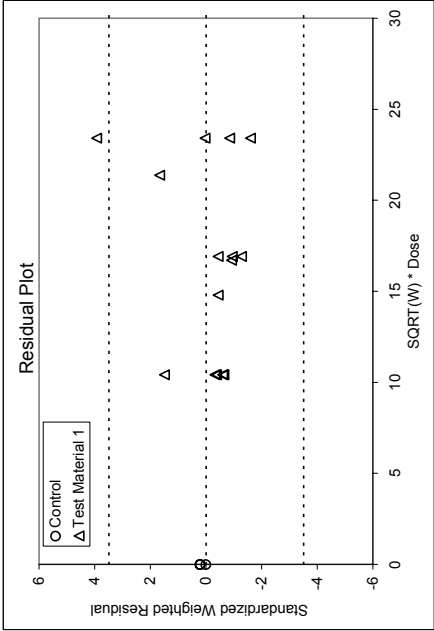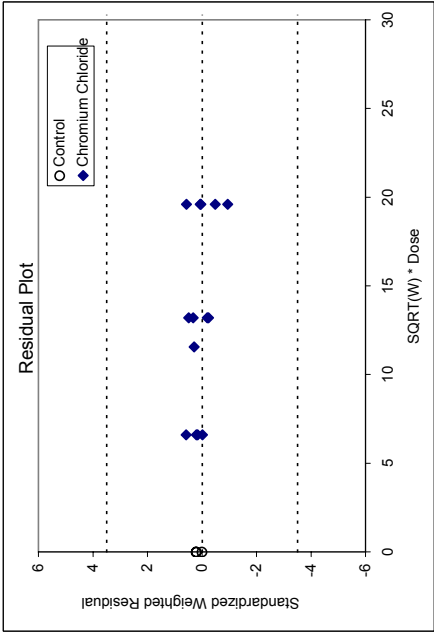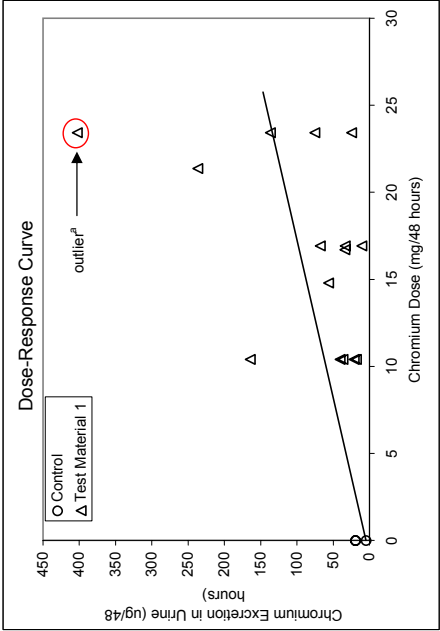
[a] Note that the data from this figure were refitted with the outlier excluded (see Figure 4-1b); this outlier was excluded from the final RBA evaluation.

### Summary of Fitting[a]

| Parameter | Estimate | Standard Error |
|---|---|---|
| $a$ | 4.94E+00 | 2.85E+01 |
| $b_r$ | 3.39E+00 | 2.26E+00 |
| $b_{t2}$ | 5.50E+00 | 1.88E+00 |
| Covariance ($b_r, b_{t2}$) | 0.6768 | -- |
| Degrees of Freedom | 30 | -- |

[a] $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

### ANOVA

| Source | MSE |
|---|---|
| Fit | 84360.64 |
| Error | 5009.26 |
| Total | 10128.70 |

| Statistic | Estimate |
|---|---|
| F | 16.841 |
| p | < 0.001 |
| Adjusted $R^2$ | 0.5054 |

### RBA and Uncertainty

| | Test Material 1 |
|---|---|
| RBA | 1.62 |
| Lower bound[b] | 0.86 |
| Upper bound[b] | -7.27 |
| Standard Error[b] | 0.817** |

[b] Calculated using Fieller's theorem

** $g \geq 0.05$, estimate is uncertain

2_Navy2 RBA Calcs_all data.xls (Graph_1) T

**FIGURE D-20  URINARY EXCRETION OF CHROMIUM:  Days 6/7 (Outliers Excluded)**
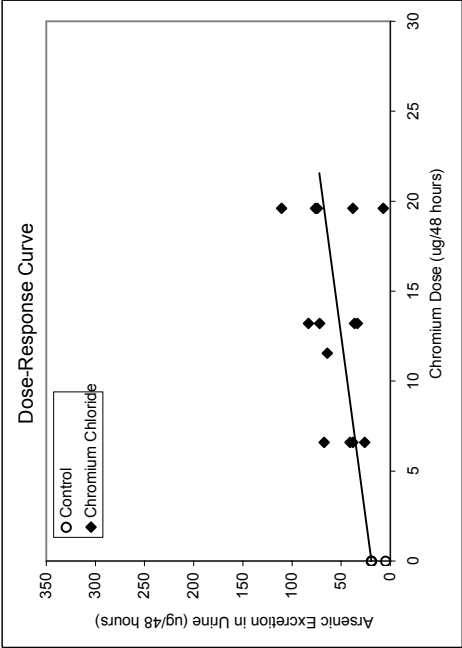
**Reference Material (Chromium Chloride)**

**Test Material 2 (McClellan Air Force Base)**



Dose-Response Curve — Reference Material (Chromium Chloride); Residual Plot; Dose-Response Curve — Test Material 2; Residual Plot.

**RBA and Uncertainty**

| | Test Material 1 |
|---|---|
| RBA | 1.27 |
| Lower bound[b] | 0.60 |
| Upper bound[b] | -16.91 |
| Standard Error[b] | 0.572** |

[b] Calculated using Fieller's theorem

** g ≥ 0.05, estimate is uncertain

**ANOVA**

| Source | MSE |
|---|---|
| Fit | 48881.13 |
| Error | 2201.01 |
| Total | 5313.02 |

| Statistic | Estimate |
|---|---|
| F | 22.209 |
| p | < 0.001 |
| Adjusted $R^2$ | 0.5857 |
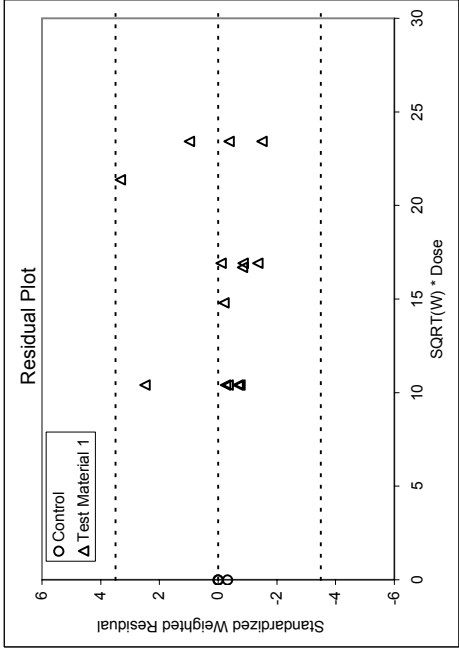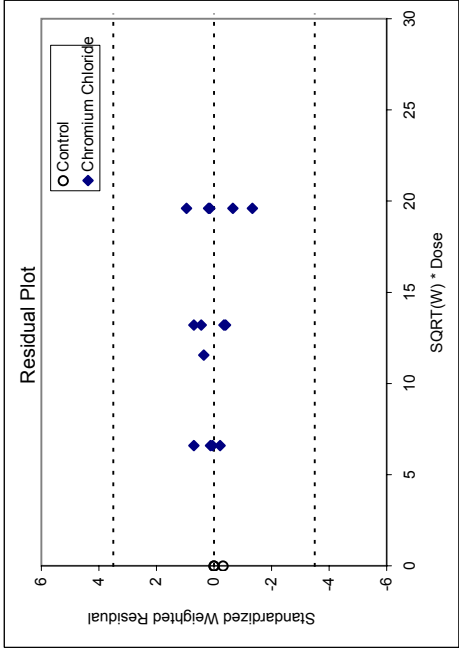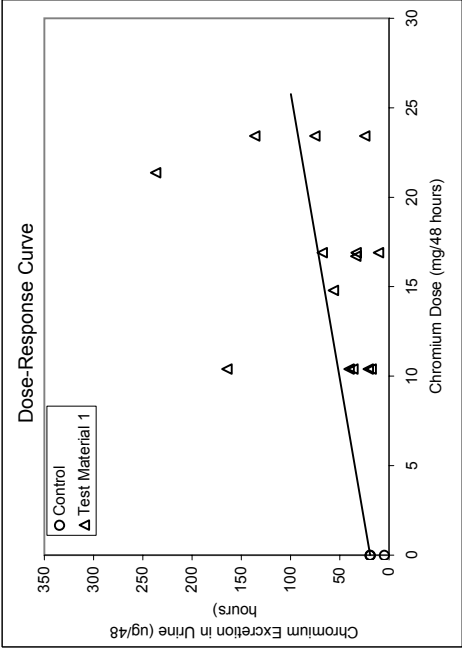
**Summary of Fitting[a]**

| Parameter | Estimate | Standard Error |
|---|---|---|
| a | 1.93E+01 | 1.91E+01 |
| $b_r$ | 2.46E+00 | 1.50E+00 |
| $b_{12}$ | 3.12E+00 | 1.31E+00 |
| Covariance ($b_r$,$b_{12}$) | 0.6728 | -- |
| Degrees of Freedom | 29 | -- |

[a] $y = a + b_r \cdot x_r + b_{12} \cdot x_{12}$

2_Navy2 RBA Calcs_outliers.xls (Graph_1)T

**FIGURE D-21  URINARY EXCRETION OF CHROMIUM:  Days 9/10 (All Data)**
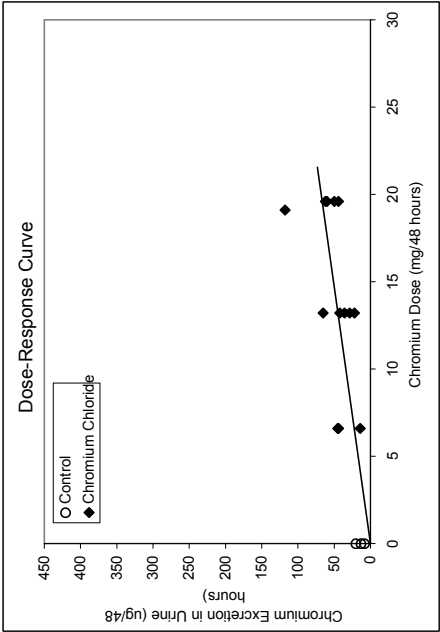
**Test Material 2 (McClellan Air Force Base)**

**Reference Material (Chromium Chloride)**
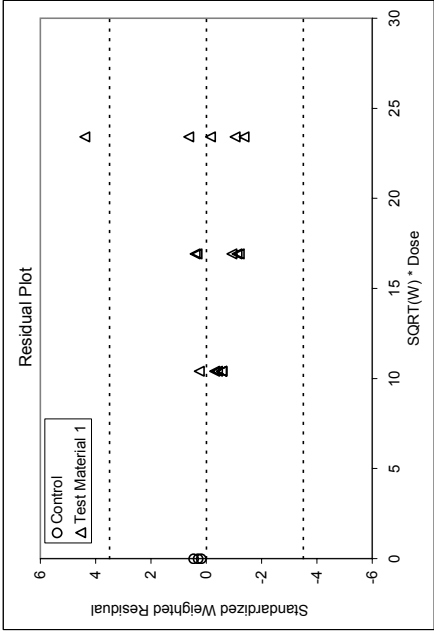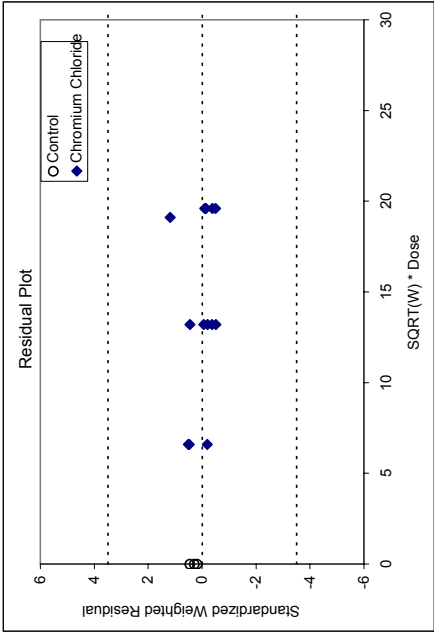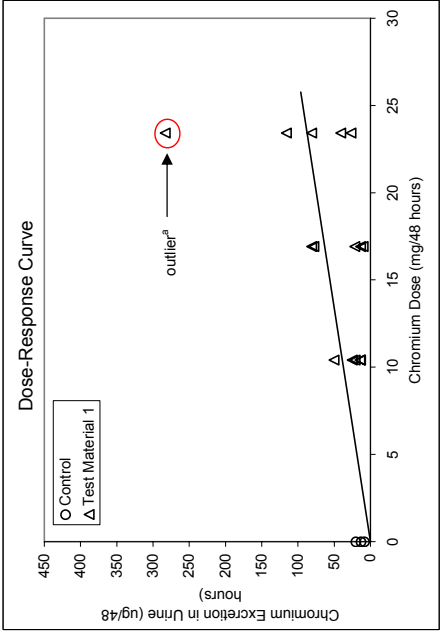


a Note that the data from this figure were refitted with the outlier excluded (see Figure 4-2b); this outlier was excluded from the final RBA evaluation.

**Summary of Fitting[a]**

| Parameter | Estimate | SE |
|---|---|---|
| a | 1.01E-01 | 1.86E+01 |
| $b_r$ | 3.40E+00 | 1.47E+00 |
| $b_{t2}$ | 3.73E+00 | 1.21E+00 |
| Covariance ($b_r, b_{t2}$) | 0.6788 | -- |
| Degrees of Freedom | 30 | -- |

a $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

**ANOVA**

| Source | MSE |
|---|---|
| Fit | 43676.28 |
| Error | 2120.72 |
| Total | 4801.72 |

| Statistic | Estimate |
|---|---|
| F | 20.595 |
| p | < 0.001 |
| Adjusted $R^2$ | 0.5583 |

**RBA and Uncertainty**

| | Test Material 1 |
|---|---|
| RBA | 1.10 |
| Lower bound[b] | 0.65 |
| Upper bound[b] | 2.81 |
| Standard Error[b] | 0.350** |

b Calculated using Fieller's theorem

** $g \geq 0.05$, estimate is uncertain

2_Navy2 RBA Calcs_all data.xls (Graph_2) T

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**FIGURE D-22  URINARY EXCRETION OF CHROMIUM:  Days 9/10 (Outliers Excluded)**

**Test Material 2 (McClellan Air Force Base)**

**Reference Material (Chromium Chloride)**



Dose-Response Curve / Residual Plot — Reference Material (Chromium Chloride) and Test Material 2 (McClellan Air Force Base)

**RBA and Uncertainty**

| | Test Material 1 |
| --- | --- |
| RBA | 0.75 |
| Lower bound[b] | 0.44 |
| Upper bound[b] | 1.18 |
| Standard Error[b] | 0.189** |

[b] Calculated using Fieller's theorem

** $g \geq 0.05$, estimate is uncertain

**ANOVA**

| Source | MSE |
| --- | --- |
| Fit | 27554.04 |
| Error | 625.41 |
| Total | 2420.65 |

| Statistic | Estimate |
| --- | --- |
| F | 44.057 |
| p | < 0.001 |
| Adjusted $R^2$ | 0.7416 |
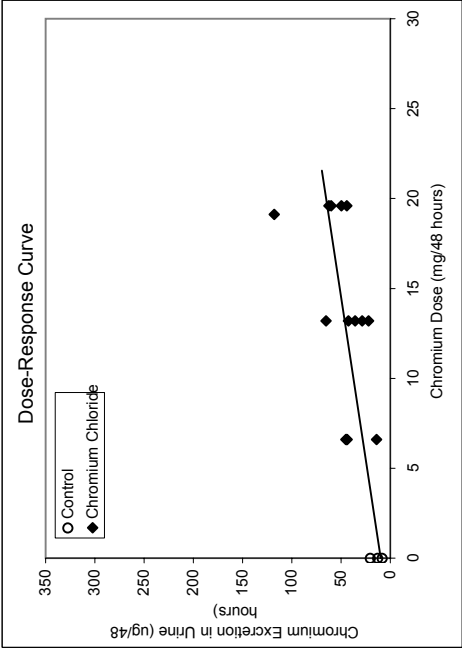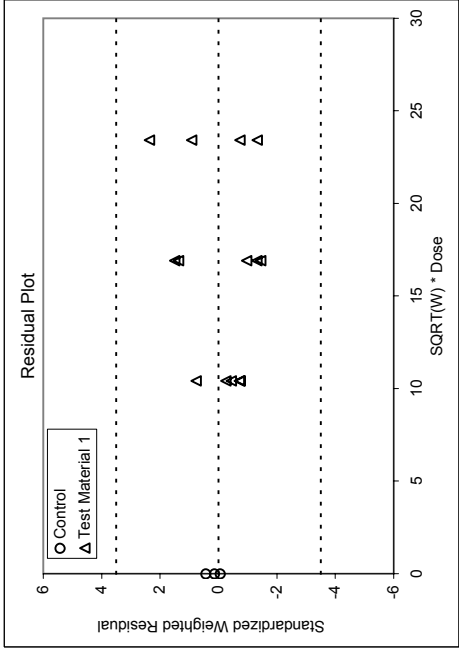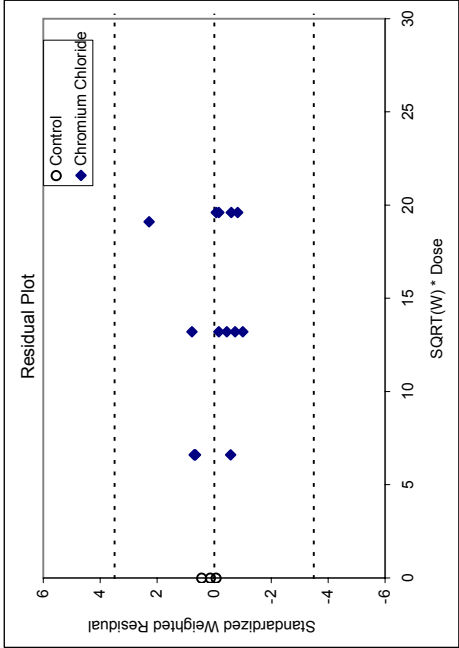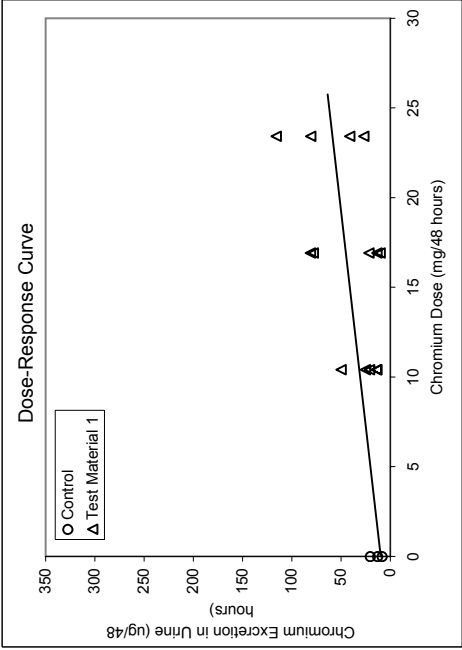
**Summary of Fitting[a]**

| Parameter | Estimate | SE |
| --- | --- | --- |
| a | 9.67E+00 | 1.02E+01 |
| $b_r$ | 2.78E+00 | 8.02E-01 |
| $b_{l2}$ | 2.10E+00 | 6.83E-01 |
| Covariance ($b_r$,$b_{l2}$) | 0.6741 | -- |
| Degrees of Freedom | 29 | -- |

[a] $y = a + b_r \cdot x_r + b_{l2} \cdot x_{l2}$

2_Navy2 RBA Calcs_outliers.xls (Graph_2)T

# FIGURE D-23 URINARY EXCRETION OF CHROMIUM: Days 12/13 (All Data)

## Test Material 2 (McClellan Air Force Base)

### Reference Material (Chromium Chloride)



Dose-Response Curve and Residual Plot for Reference Material (Chromium Chloride) and Test Material 2
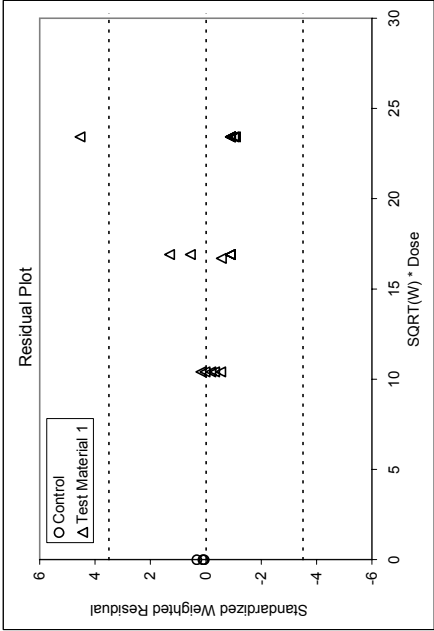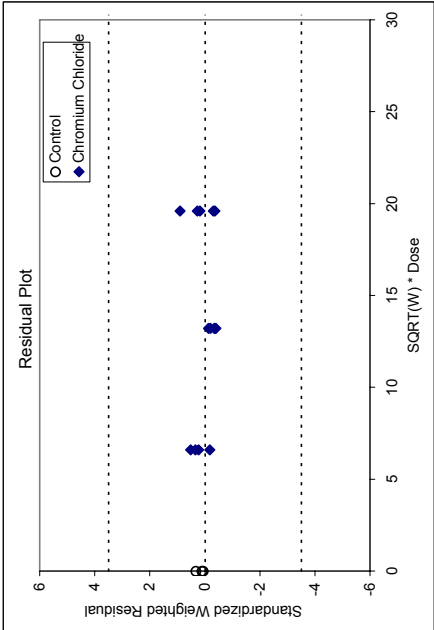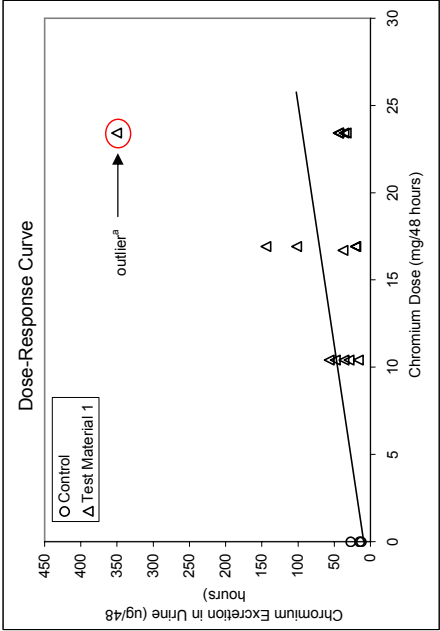
[a] Note that the data from this figure were refitted with the outlier excluded (see Figure 4-3b); this outlier was excluded from the final RBA evaluation.

## RBA and Uncertainty

| | Test Material 1 |
|---|---|
| RBA | 1.32 |
| Lower bound[b] | 0.62 |
| Upper bound[b] | -4.42 |
| Standard Error[b] | 0.656** |

[b] Calculated using Fieller's theorem

** $g \geq 0.05$, estimate is uncertain

## ANOVA

| Source | MSE |
|---|---|
| Fit | 49358.54 |
| Error | 3394.64 |
| Total | 6360.05 |

| Statistic | Estimate |
|---|---|
| F | 14.540 |
| p | < 0.001 |
| Adjusted $R^2$ | 0.4663 |

## Summary of Fitting[a]

| Parameter | Estimate | SE |
|---|---|---|
| a | 9.26E+00 | 2.35E+01 |
| $b_r$ | 2.75E+00 | 1.85E+00 |
| $b_{t2}$ | 3.62E+00 | 1.53E+00 |
| Covariance $(b_r, b_{t2})$ | 0.6780 | -- |
| Degrees of Freedom | 30 | -- |

[a] $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

2_Navy2 RBA Calcs_all data.xls (Graph_3) T

# FIGURE D-24  URINARY EXCRETION OF CHROMIUM:  Days 12/13 (Outliers Excluded)

## Reference Material (Chromium Chloride)

## Test Material 2 (McClellan Air Force Base)



Dose-Response Curve — Reference Material (Chromium Chloride)



Dose-Response Curve — Test Material 2



Residual Plot — Reference Material



Residual Plot — Test Material

### RBA and Uncertainty

| | Test Material 1 |
|---|---|
| RBA | 0.77 |
| Lower bound[b] | 0.16 |
| Upper bound[b] | 2.06 |
| Standard Error[b] | 0.312** |

[b] Calculated using Fieller's theorem

** $g \geq 0.05$, estimate is uncertain

### ANOVA

| Source | MSE |
|---|---|
| Fit | 28300.32 |
| Error | 824.39 |
| Total | 2656.12 |

| Statistic | Estimate |
|---|---|
| F | 34.329 |
| p | < 0.001 |
| Adjusted $R^2$ | 0.6896 |
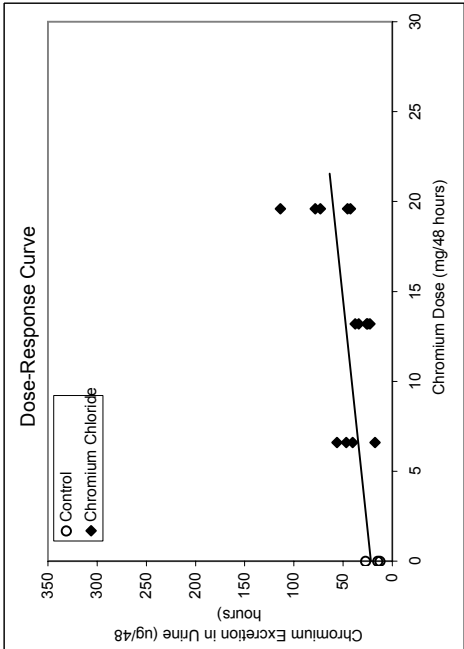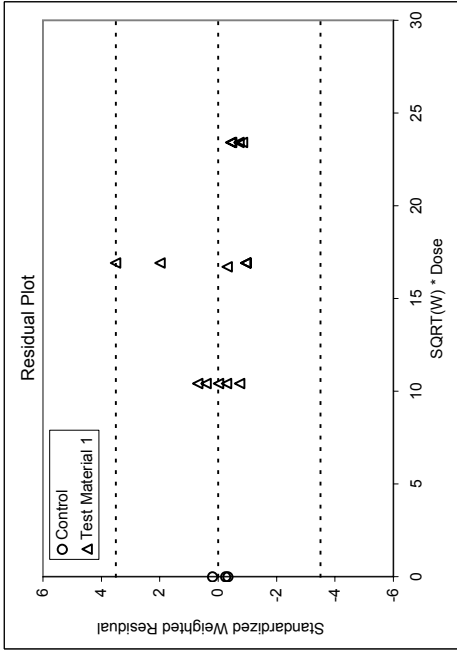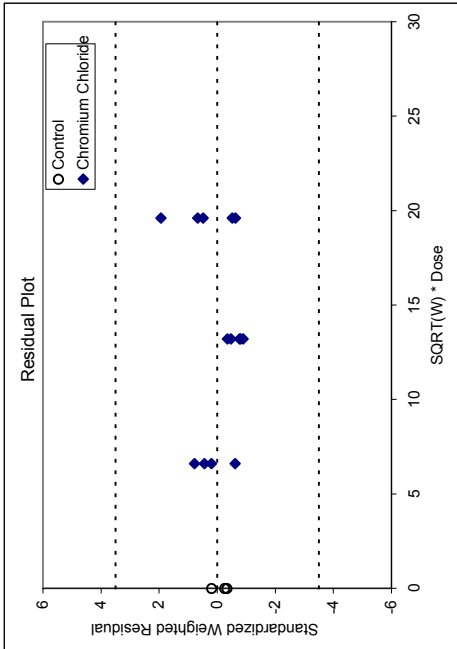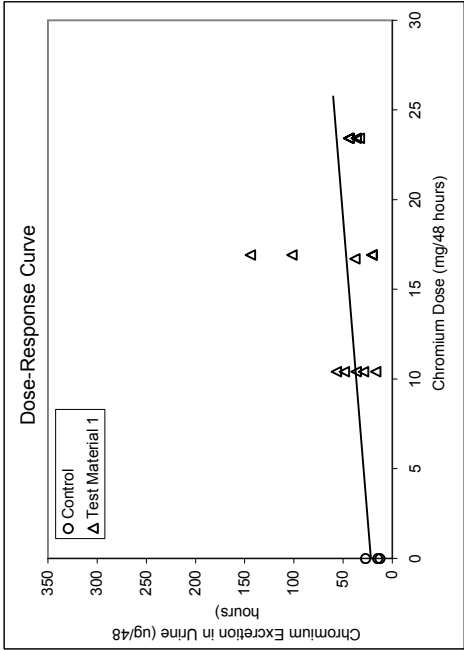
### Summary of Fitting[a]

| Parameter | Estimate | SE |
|---|---|---|
| a | 2.18E+01 | 1.17E+01 |
| $b_r$ | 1.94E+00 | 9.16E-01 |
| $b_{t2}$ | 1.49E+00 | 7.84E-01 |
| Covariance ($b_r,b_{t2}$) | 0.6733 | -- |
| Degrees of Freedom | 29 | -- |

[a] $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

2_Navy2 RBA Calcs_outliers.xls (Graph_3)T

# FIGURE D-25 URINARY EXCRETION OF CHROMIUM: All Days (All Data)

## Reference Material (Chromium Chloride)
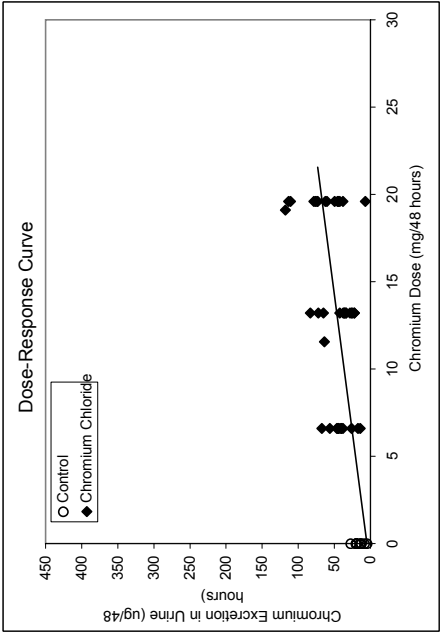
## Test Material 2 (McClellan Air Force Base)
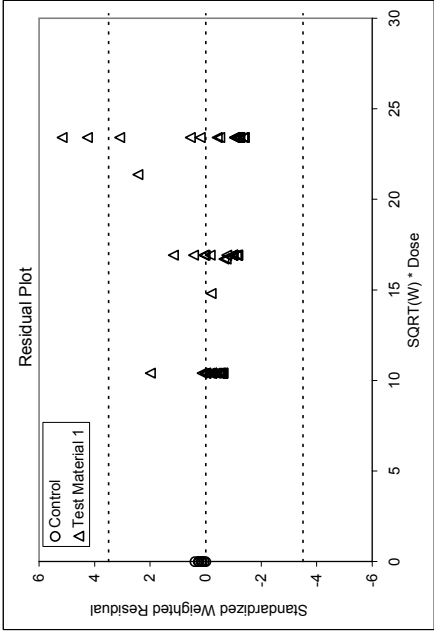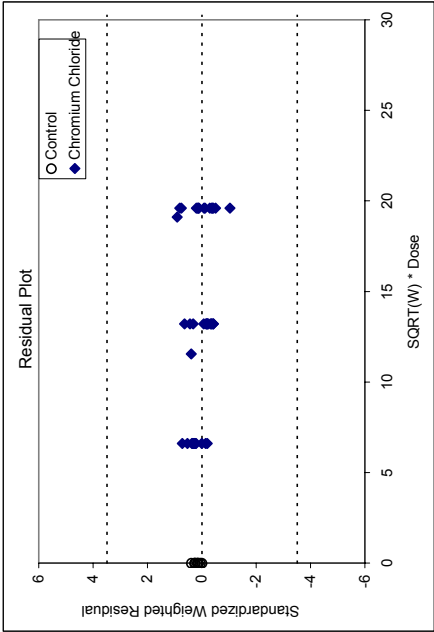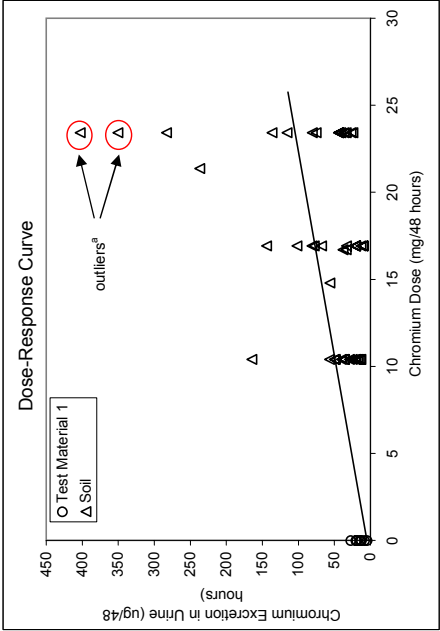


[a] Note that the data from this figure were refitted with the outliers excluded (see Figure 4-4b); these outliers were excluded from the final RBA evaluation.

## Summary of Fitting[b]

| Parameter | Estimate | SE |
|---|---|---|
| a | 5.08E+00 | 1.36E+01 |
| $b_r$ | 3.15E+00 | 1.07E+00 |
| $b_{t2}$ | 4.25E+00 | 8.87E-01 |
| Covariance ($b_r,b_{t2}$) | 0.6778 | -- |
| Degrees of Freedom | 94 | -- |

[a] $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

## ANOVA

| Source | MSE |
|---|---|
| Fit | 128993.74 |
| Error | 3402.52 |
| Total | 6046.55 |

| Statistic | Estimate |
|---|---|
| F | 37.911 |
| p | < 0.001 |
| Adjusted $R^2$ | 0.4373 |

## RBA and Uncertainty

| | Test Material 1 |
|---|---|
| RBA | 1.35 |
| Lower bound[c] | 0.94 |
| Upper bound[c] | 2.49 |
| Standard Error[c] | 0.338** |

[c] Calculated using Fieller's theorem

** $g \geq 0.05$, estimate is uncertain

2_Navy2 RBA Calcs_all data.xls (Graph_All) T

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**FIGURE D-26 URINARY EXCRETION OF CHROMIUM: All Days (Outliers Excluded)**

**Test Material 2 (McClellan Air Force Base)**

**Reference Material (Chromium Chloride)**



Dose-Response Curve

Residual Plot

**RBA and Uncertainty**

| | Test Material 1 |
|---|---|
| RBA | 1.07 |
| Lower bound[c] | 0.76 |
| Upper bound[c] | 1.69 |
| Standard Error[c] | 0.234** |

[c] Calculated using Fieller's theorem

** g ≥ 0.05, estimate is uncertain

**ANOVA**

| Source | MSE |
|---|---|
| Fit | 98889.66 |
| Error | 1436.52 |
| Total | 2484.40 |

| Statistic | Estimate |
|---|---|
| F | 68.840 |
| p | < 0.001 |
| Adjusted R² | 0.4218 |
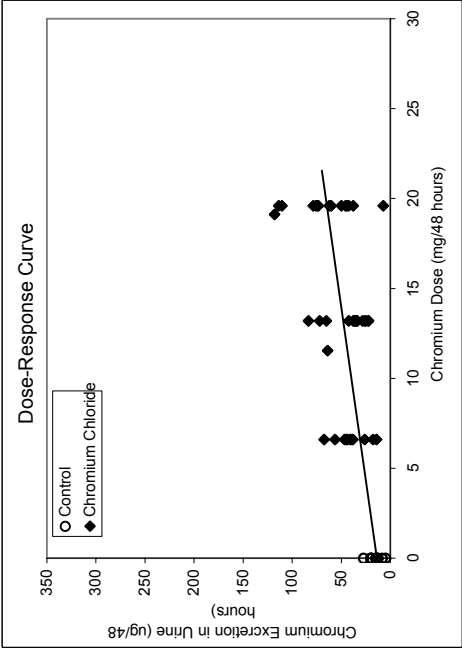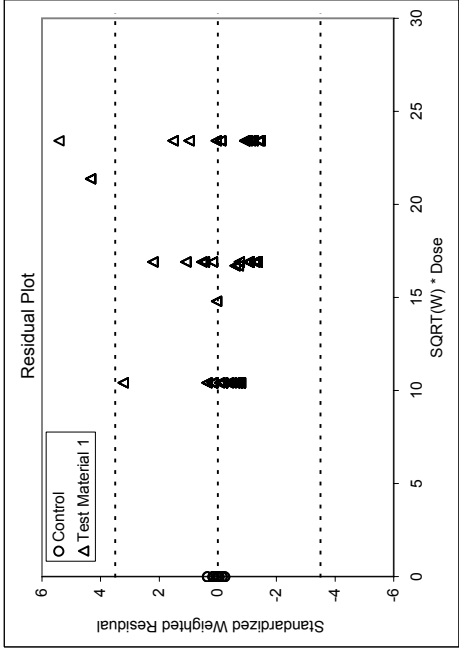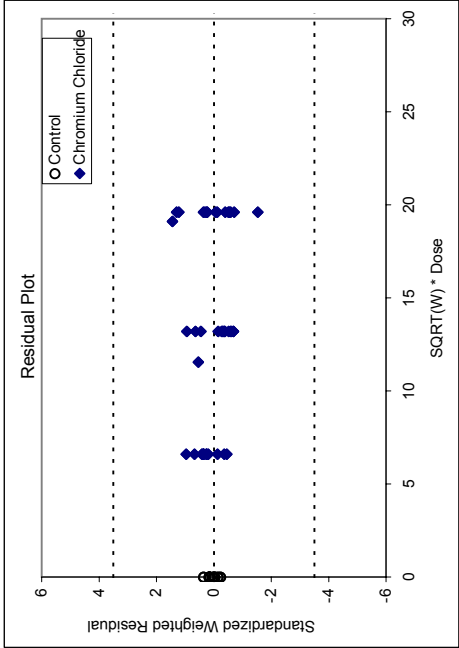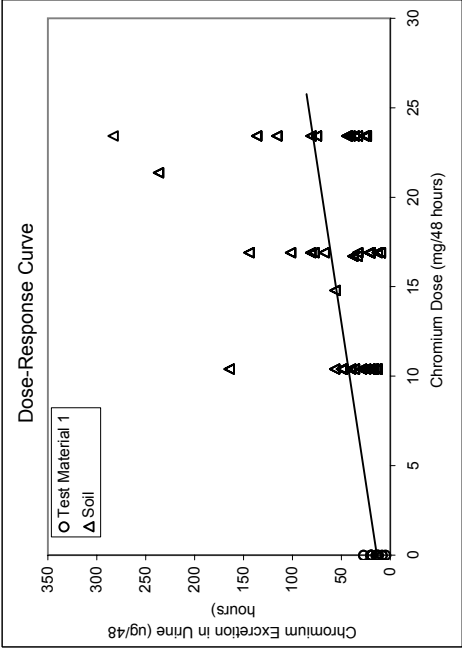
**Summary of Fitting[b]**

| Parameter | Estimate | SE |
|---|---|---|
| a | 1.38E+01 | 9.60E+00 |
| $b_r$ | 2.60E+00 | 7.57E-01 |
| $b_{t2}$ | 2.78E+00 | 6.41E-01 |
| Covariance ($b_r$,$b_{t2}$) | 0.6748 | -- |
| Degrees of Freedom | 185 | -- |

[a] $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

2_Navy2 RBA Calcs_outliers.xls (Graph_All)T

**TABLE D-16 SCHEDULE**

| Study Day | Day | Date | Feed Special Diet | Cull Pigs/ Assign Dose Group | Weigh | Dose Preparation | Dose Administration | Bleed | Urine Collection[a] | Sacrifice/ Necropsy |
|---|---|---|---|---|---|---|---|---|---|---|
| -5 | Wednesday | 10/25/06 | | Cull Pigs | X | | | | | |
| -4 | Thursday | 10/26/06 | transition | Assign Dose Groups | | | | | | |
| -3 | Friday | 10/27/06 | transition | | | | | | | |
| -2 | Saturday | 10/28/06 | transition | | | | | | | |
| -1 | Sunday | 10/29/06 | transition | | X | X | | | | |
| 0 | Monday | 10/30/06 | X | | | | X | X | | |
| 1 | Tuesday | 10/31/06 | X | | | | X | | | |
| 2 | Wednesday | 11/01/06 | X | | X | | X | | | |
| 3 | Thursday | 11/02/06 | X | | | X | X | | | |
| 4 | Friday | 11/03/06 | X | | | | X | | | |
| 5 | Saturday | 11/04/06 | X | | X | | X | | | |
| 6 | Sunday | 11/05/06 | X | | | | X | | U-1 | |
| 7 | Monday | 11/06/06 | X | | | | X | | | |
| 8 | Tuesday | 11/07/06 | X | | X | X | X | X | | |
| 9 | Wednesday | 11/08/06 | X | | | | X | | U-2 | |
| 10 | Thursday | 11/09/06 | X | | | | X | | | |
| 11 | Friday | 11/10/06 | X | | X | X | X | | | |
| 12 | Saturday | 11/11/06 | X | | | | X | | U-3 | |
| 13 | Sunday | 11/12/06 | X | | | | X | | | |
| 14 | Monday | 11/13/06 | | | X | | | X | | X |

[a] Urine was collected over a period of 48 hours.

Navy2_Appendix A.xls (A-1_Schedule)

# TABLE D-17  GROUP ASSIGNMENTS

| Pig Number | Dose Group | Material Administered | Target Dose of Chromium (µg/kg-day) |
|---|---|---|---|
| 208<br>210<br>212<br>219<br>234 | 1 | Chromium Chloride | 250 |
| 201<br>205<br>221<br>229<br>232 | 2 | Chromium Chloride | 500 |
| 203<br>204<br>209<br>211<br>240 | 3 | Chromium Chloride | 750 |
| 214<br>224<br>231<br>235<br>242 | 4 | Test Material 2 | 400 |
| 220<br>222<br>226<br>228<br>241 | 5 | Test Material 2 | 650 |
| 202<br>207<br>217<br>223<br>225 | 6 | Test Material 2 | 900 |
| 227<br>233<br>237 | 7 | Control | 0 |

# TABLE D-18  BODY WEIGHTS AND ACTUAL ADMINISTERED DOSES, BY DAY

Body weights were measured on days -1, 2, 5, 8, 11, and 14.  Weights for other days are estimated, based on linear interpolation between measured values.

| Group | Pig # | Day -1 BW (kg) | Day -1 As Dose (µg/kg-d) | Day 0 BW (kg) | Day 0 As Dose | Day 1 BW (kg) | Day 1 As Dose | Day 2 BW (kg) | Day 2 As Dose | Day 3 BW (kg) | Day 3 As Dose | Day 4 BW (kg) | Day 4 As Dose | Day 5 BW (kg) | Day 5 As Dose | Day 6 BW (kg) | Day 6 As Dose | Day 7 BW (kg) | Day 7 As Dose | Day 8 BW (kg) | Day 8 As Dose | Day 9 BW (kg) | Day 9 As Dose | Day 10 BW (kg) | Day 10 As Dose | Day 11 BW (kg) | Day 11 As Dose | Day 12 BW (kg) | Day 12 As Dose | Day 13 BW (kg) | Day 13 As Dose | Day 14 BW (kg) | Day 14 As Dose | Days 0-14 Mean As Dose (µg/kg-d) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 208 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | 210 | 9.4 | 0.00 | 9.8 | 368.60 | 10.1 | 355.26 | 10.5 | 342.86 | 10.7 | 336.97 | 10.9 | 303.68 | 11.1 | 298.64 | 11.4 | 289.05 | 11.8 | 280.06 | 12.2 | 271.60 | 12.5 | 264.00 | 12.9 | 256.81 | 13.2 | 250.00 | 13.6 | 242.94 | 14.0 | 236.28 | 14.4 | 229.97 | 296.33 |
| 1 | 212 | 8.3 | 0.00 | 8.7 | 416.18 | 9.1 | 397.79 | 9.5 | 380.95 | 9.8 | 367.97 | 10.1 | 326.19 | 10.5 | 315.79 | 11.1 | 296.41 | 11.8 | 279.27 | 12.5 | 264.00 | 12.6 | 261.56 | 12.7 | 259.16 | 12.9 | 256.81 | 13.2 | 250.63 | 13.5 | 244.75 | 13.8 | 239.13 | 314.92 |
| 1 | 219 | 9.7 | 0.00 | 9.8 | 366.72 | 10.0 | 360.60 | 10.2 | 354.68 | 10.5 | 344.50 | 10.8 | 306.98 | 11.1 | 298.64 | 11.4 | 289.90 | 11.7 | 281.65 | 12.1 | 273.86 | 12.9 | 264.71 | 12.9 | 256.14 | 13.3 | 248.12 | 13.8 | 240.00 | 14.2 | 232.39 | 14.7 | 225.26 | 297.93 |
| 1 | 234 | 8.4 | 0.00 | 8.8 | 411.43 | 9.2 | 393.44 | 9.6 | 376.96 | 9.9 | 365.48 | 10.2 | 325.12 | 10.5 | 315.79 | 10.8 | 306.50 | 11.1 | 297.74 | 11.4 | 289.47 | 11.8 | 279.27 | 12.2 | 269.75 | 12.7 | 260.87 | 13.0 | 253.20 | 13.4 | 245.96 | 13.8 | 239.13 | 318.35 |
| 2 | 201 | 8.5 | 0.00 | 8.9 | 744.36 | 9.2 | 714.80 | 9.6 | 687.50 | 9.9 | 665.55 | 10.2 | 644.95 | 10.6 | 625.59 | 10.9 | 604.58 | 11.3 | 584.93 | 11.7 | 566.52 | 12.0 | 549.24 | 12.4 | 532.97 | 12.8 | 517.65 | 13.2 | 500.63 | 13.6 | 484.70 | 14.1 | 469.75 | 607.82 |
| 2 | 205 | 9.1 | 0.00 | 9.3 | 707.14 | 9.6 | 689.90 | 9.8 | 673.47 | 10.0 | 657.81 | 10.3 | 642.86 | 10.5 | 628.57 | 10.9 | 607.36 | 11.2 | 587.54 | 11.6 | 568.97 | 12.0 | 550.76 | 12.4 | 533.69 | 12.8 | 517.65 | 13.1 | 503.18 | 13.5 | 489.49 | 13.9 | 476.53 | 602.73 |
| 2 | 221 | 9.1 | 0.00 | 9.3 | 713.51 | 9.5 | 698.41 | 9.7 | 512.95 | 9.8 | 674.62 | 9.9 | 632.27 | 10.1 | 656.72 | 10.4 | 475.96 | 10.8 | 613.95 | 11.1 | 594.59 | 11.5 | 574.75 | 11.9 | 556.18 | 12.3 | 538.78 | 12.7 | 520.37 | 13.1 | 503.18 | 13.6 | 487.08 | 590.30 |
| 2 | 229 | 9.9 | 0.00 | 10.2 | 646.00 | 10.6 | 623.62 | 11.0 | 602.74 | 11.2 | 587.54 | 11.5 | 573.08 | 11.8 | 559.32 | 12.2 | 542.47 | 12.5 | 526.60 | 12.9 | 511.63 | 13.1 | 503.18 | 13.3 | 495.00 | 13.6 | 487.08 | 14.0 | 471.99 | 14.4 | 457.80 | 14.9 | 444.44 | 545.60 |
| 2 | 232 | 9.6 | 0.00 | 9.7 | 683.94 | 9.7 | 680.41 | 9.8 | 676.92 | 9.9 | 668.92 | 10.0 | 661.10 | 10.1 | 653.47 | 10.3 | 640.78 | 10.5 | 628.57 | 10.7 | 616.82 | 11.1 | 596.39 | 11.4 | 577.26 | 11.8 | 559.32 | 12.3 | 538.04 | 12.7 | 518.32 | 13.2 | 500.00 | 622.27 |
| 3 | 203 | 9.5 | 0.00 | 9.7 | 1006.85 | 10.0 | 978.37 | 10.3 | 951.46 | 10.7 | 920.19 | 11.0 | 890.91 | 11.4 | 863.44 | 11.7 | 836.42 | 12.1 | 811.03 | 12.5 | 787.15 | 12.9 | 757.73 | 13.4 | 730.43 | 13.9 | 705.04 | 14.4 | 680.56 | 14.9 | 657.72 | 15.4 | 636.36 | 835.04 |
| 3 | 204 | 9.5 | 0.00 | 9.7 | 1010.31 | 10.0 | 984.92 | 10.2 | 960.78 | 10.5 | 936.31 | 10.7 | 913.04 | 11.0 | 890.91 | 11.3 | 864.71 | 11.7 | 840.00 | 12.0 | 816.67 | 12.4 | 790.32 | 12.8 | 765.63 | 13.2 | 742.42 | 13.6 | 720.59 | 14.0 | 700.00 | 14.4 | 680.56 | 859.90 |
| 3 | 209 | 9.2 | 0.00 | 9.6 | 1020.83 | 10.1 | 975.12 | 10.5 | 933.33 | 10.8 | 906.01 | 11.1 | 880.24 | 11.5 | 855.90 | 11.8 | 834.04 | 12.1 | 813.28 | 12.4 | 793.52 | 13.0 | 755.78 | 13.6 | 721.47 | 14.2 | 690.14 | 14.6 | 669.70 | 15.1 | 650.44 | 15.5 | 632.26 | 830.39 |
| 3 | 211 | 8.7 | 0.00 | 9.0 | 1084.87 | 9.4 | 1046.26 | 9.7 | 1010.31 | 10.0 | 981.64 | 10.3 | 954.55 | 10.6 | 928.91 | 10.8 | 910.22 | 11.0 | 892.26 | 11.2 | 875.00 | 11.6 | 842.41 | 12.1 | 771.55 | 12.5 | 784.00 | 13.0 | 756.76 | 13.4 | 731.34 | 13.9 | 707.58 | 906.64 |
| 3 | 240 | 8.9 | 0.00 | 9.3 | 1059.46 | 9.6 | 1020.83 | 10.0 | 984.92 | 10.1 | 970.30 | 10.3 | 956.10 | 10.4 | 942.31 | 10.7 | 917.32 | 11.0 | 893.62 | 11.3 | 871.11 | 11.7 | 841.20 | 12.1 | 813.28 | 12.5 | 787.15 | 12.9 | 759.69 | 13.4 | 734.08 | 13.8 | 710.14 | 901.66 |
| 4 | 214 | 10.0 | 0.00 | 10.4 | 500.34 | 10.9 | 467.60 | 11.3 | 460.49 | 11.7 | 446.66 | 12.0 | 433.63 | 12.4 | 421.34 | 12.7 | 410.81 | 13.0 | 400.79 | 13.3 | 391.24 | 13.1 | 398.74 | 12.8 | 406.53 | 12.6 | 414.63 | 13.7 | 381.21 | 14.8 | 352.78 | 15.9 | 328.30 | 416.27 |
| 4 | 224 | 10.0 | 0.00 | 10.3 | 504.38 | 10.7 | 487.07 | 11.1 | 470.91 | 11.4 | 457.79 | 11.7 | 445.38 | 12.0 | 433.63 | 12.4 | 420.77 | 12.7 | 408.66 | 13.1 | 397.22 | 13.7 | 381.21 | 14.2 | 366.45 | 14.8 | 352.78 | 15.1 | 345.37 | 15.4 | 338.26 | 15.7 | 331.44 | 420.07 |
| 4 | 231 | 8.6 | 0.00 | 9.0 | 579.24 | 9.4 | 552.59 | 9.9 | 528.28 | 10.2 | 509.32 | 10.6 | 491.67 | 11.0 | 475.21 | 11.4 | 456.45 | 11.9 | 439.12 | 12.3 | 423.05 | 12.7 | 409.19 | 13.1 | 396.21 | 13.6 | 384.03 | 14.1 | 369.05 | 14.7 | 355.19 | 15.2 | 342.34 | 460.13 |
| 4 | 235 | 9.5 | 0.00 | 9.9 | 527.39 | 10.2 | 508.49 | 10.6 | 490.90 | 11.0 | 473.77 | 11.4 | 457.79 | 11.8 | 442.86 | 12.2 | 428.28 | 12.6 | 414.63 | 13.0 | 401.82 | 13.5 | 386.88 | 14.0 | 373.01 | 14.5 | 360.11 | 14.9 | 349.23 | 15.4 | 338.99 | 15.8 | 329.34 | 430.29 |
| 4 | 242 | 10.2 | 0.00 | 10.3 | 504.38 | 10.5 | 446.73 | 10.7 | 366.45 | 11.1 | 448.79 | 11.6 | 450.52 | 12.0 | 433.63 | 12.4 | 419.64 | 12.8 | 406.53 | 13.2 | 394.21 | 13.7 | 380.75 | 14.1 | 368.18 | 14.6 | 356.41 | 15.2 | 342.71 | 15.8 | 330.03 | 16.4 | 318.26 | 406.82 |
| 5 | 220 | 9.9 | 0.00 | 10.1 | 835.80 | 10.3 | 818.27 | 10.6 | 801.47 | 10.8 | 743.77 | 11.0 | 650.42 | 11.3 | 748.27 | 11.7 | 541.25 | 12.1 | 696.88 | 12.6 | 673.74 | 13.0 | 648.76 | 13.5 | 625.56 | 14.0 | 603.96 | 14.2 | 596.86 | 14.3 | 589.92 | 14.5 | 583.14 | 689.98 |
| 5 | 222 | 8.8 | 0.00 | 9.2 | 922.42 | 9.5 | 886.94 | 9.9 | 854.09 | 10.2 | 826.27 | 10.6 | 800.20 | 10.9 | 775.73 | 11.3 | 748.27 | 11.7 | 722.69 | 12.1 | 698.80 | 12.7 | 680.98 | 13.1 | 664.04 | 13.4 | 647.93 | 13.8 | 631.01 | 14.1 | 614.94 | 14.5 | 599.68 | 756.75 |
| 5 | 226 | 9.5 | 0.00 | 9.5 | 890.05 | 9.8 | 862.80 | 10.1 | 837.18 | 10.5 | 806.56 | 10.9 | 778.11 | 11.3 | 751.60 | 11.6 | 732.08 | 11.9 | 713.54 | 12.2 | 695.92 | 13.2 | 667.54 | 13.2 | 641.38 | 13.7 | 617.19 | 14.0 | 602.53 | 14.4 | 588.55 | 14.7 | 575.20 | 736.18 |
| 5 | 228 | 9.5 | 0.00 | 9.9 | 858.42 | 10.2 | 828.97 | 10.6 | 801.47 | 10.9 | 773.37 | 11.3 | 747.17 | 11.7 | 722.69 | 12.1 | 699.76 | 12.5 | 678.25 | 12.9 | 658.01 | 13.3 | 638.15 | 13.7 | 619.45 | 14.1 | 601.81 | 14.4 | 587.19 | 14.8 | 558.92 | 15.1 | 559.97 | 706.18 |
| 5 | 241 | 8.8 | 0.00 | 9.1 | 927.47 | 9.5 | 853.75 | 9.9 | 858.42 | 10.3 | 826.27 | 10.6 | 796.43 | 11.0 | 768.68 | 11.3 | 750.49 | 11.8 | 714.81 | 12.2 | 716.57 | 12.7 | 691.18 | 12.7 | 667.54 | 13.1 | 645.46 | 13.4 | 629.44 | 13.8 | 614.20 | 14.1 | 599.68 | 757.84 |
| 6 | 202 | 9.2 | 0.00 | 9.4 | 1121.03 | 9.6 | 1168.91 | 9.8 | 925.93 | 10.1 | 1128.42 | 10.4 | 1122.23 | 10.6 | 1089.17 | 11.0 | 935.62 | 11.4 | 997.59 | 11.6 | 1031.59 | 11.7 | 999.31 | 12.1 | 968.99 | 12.5 | 940.45 | 12.9 | 908.82 | 13.3 | 879.24 | 13.8 | 851.53 | 987.08 |
| 6 | 207 | 9.3 | 0.00 | 9.6 | 1215.42 | 10.4 | 1106.32 | 10.4 | 1125.83 | 10.6 | 1101.12 | 10.9 | 1077.48 | 11.1 | 1054.83 | 11.4 | 1030.08 | 11.9 | 1006.47 | 12.3 | 983.91 | 12.6 | 955.80 | 13.0 | 929.25 | 13.3 | 904.14 | 13.6 | 882.56 | 13.9 | 861.98 | 13.9 | 842.34 | 1029.24 |
| 6 | 217 | 9.8 | 0.00 | 10.2 | 1151.66 | 10.6 | 1106.32 | 11.0 | 1064.42 | 11.3 | 1034.63 | 11.6 | 1006.47 | 11.7 | 979.80 | 12.3 | 954.50 | 12.6 | 930.48 | 12.9 | 907.64 | 13.3 | 881.45 | 13.7 | 856.73 | 14.4 | 833.35 | 14.4 | 814.04 | 14.7 | 795.60 | 15.1 | 777.98 | 960.30 |
| 6 | 223 | 10.0 | 0.00 | 10.3 | 1136.76 | 10.6 | 1049.35 | 10.9 | 1074.18 | 11.2 | 1048.53 | 11.4 | 1024.07 | 11.7 | 1000.73 | 12.1 | 970.32 | 12.4 | 941.71 | 12.8 | 914.73 | 13.2 | 885.90 | 13.6 | 858.82 | 14.1 | 833.35 | 14.4 | 812.16 | 14.8 | 792.01 | 15.2 | 772.84 | 961.45 |
| 6 | 225 | 9.4 | 0.00 | 9.6 | 1223.89 | 9.8 | 1196.79 | 10.0 | 1170.86 | 10.2 | 1146.03 | 10.4 | 1122.23 | 10.7 | 1099.40 | 11.0 | 1069.28 | 11.3 | 1040.76 | 11.6 | 1013.73 | 11.9 | 981.17 | 12.3 | 950.63 | 12.7 | 921.94 | 13.0 | 900.66 | 13.3 | 880.34 | 13.6 | 860.93 | 1060.41 |
| 7 | 227 | 8.4 | 0.00 | 8.7 | 0.00 | 9.1 | 0.00 | 9.4 | 0.00 | 10.0 | 0.00 | 10.6 | 0.00 | 11.2 | 0.00 | 11.3 | 0.00 | 11.4 | 0.00 | 11.6 | 0.00 | 12.0 | 0.00 | 12.4 | 0.00 | 12.8 | 0.00 | 13.2 | 0.00 | 13.5 | 0.00 | 13.9 | 0.00 | 0.00 |
| 7 | 233 | 9.0 | 0.00 | 9.3 | 0.00 | 9.7 | 0.00 | 10.0 | 0.00 | 10.4 | 0.00 | 10.7 | 0.00 | 11.1 | 0.00 | 11.4 | 0.00 | 11.7 | 0.00 | 12.0 | 0.00 | 12.2 | 0.00 | 12.5 | 0.00 | 12.8 | 0.00 | 13.2 | 0.00 | 13.7 | 0.00 | 14.2 | 0.00 | 0.00 |
| 7 | 237 | 9.2 | 0.00 | 9.5 | 0.00 | 9.8 | 0.00 | 10.1 | 0.00 | 10.5 | 0.00 | 10.9 | 0.00 | 11.3 | 0.00 | 11.7 | 0.00 | 12.0 | 0.00 | 12.4 | 0.00 | 12.8 | 0.00 | 13.2 | 0.00 | 13.6 | 0.00 | 14.0 | 0.00 | 14.5 | 0.00 | 14.9 | 0.00 | 0.00 |

**Missed Doses:**

Day 0 - Pig 202 did not eat entire PM dose (ate approximately 80%).  Daily dose adjusted to 90%.
Day 1 - Pig 202 did not eat entire AM or PM dose (ate approximately 90% and 50%, respectively).  Daily dose adjusted to 70%.
Day 1 - Pig 223 did not eat entire AM dose (ate approximately 90%).  Daily dose adjusted to 95%.
Day 1 - Pig 242 did not eat entire AM dose (ate approximately 80%).  Daily dose adjusted to 90%.
Day 1 - Pig 214 did not eat entire PM dose (ate approximately 95%).  Daily dose adjusted to 97.5%.
Day 2 - Pig 202 did not eat entire AM or PM dose (ate approximately 60% and 75%, respectively).  Daily dose adjusted to 78%.
Day 2 - Pig 242 did not eat entire AM or PM dose (ate approximately 50%).  Daily dose adjusted to 75%.
Day 2 - Pig 221 did not eat entire AM or PM dose (ate approximately 50%).  Daily dose adjusted to 75%.
Day 3 - Pig 202 did not eat entire PM dose (ate approximately 95%).  Daily dose adjusted to 97.5%.
Day 3 - Pig 220 did not eat entire PM dose (ate approximately 90%).  Daily dose adjusted to 95%.

Day 4 - Pig 221 did not eat entire PM dose (ate approximately 90%)  Daily dose adjusted to 95%.
Day 4 - Pig 220 did not eat entire PM dose (ate approximately 70%).  Daily dose adjusted to 85%.
Day 5 - Pig 220 did not eat entire PM dose; unseaten dose was not quantified, so daily dose could not be adjusted.
Day 6 - Pig 202 did not eat entire PM dose (ate approximately 75%).  Daily dose adjusted to 87.5%.
Day 6 - Pig 221 did not eat entire PM dose (ate approximately 50%).  Daily dose adjusted to 75%.
Day 6 - Pig 220 did not eat entire AM or PM dose (ate approximately 50%).  Daily dose adjusted to 75%.
Day 7 - Pig 202 did not eat entire PM dose (ate approximately 90%).  Daily dose adjusted to 95%.
Day 7 - Pig 241 did not eat entire AM dose (ate approximately 95%).  Daily dose adjusted to 97.5%.
Day 10 - Pig 211 did not eat entire AM dose (ate approximately 90%).  Daily dose adjusted to 95%.
Day 13 - Pig 228 did not eat entire AM dose (ate approximately 95%).  Daily dose adjusted to 97.5%.

Instances of late consumption of doses are shown in Table A-5 (no adjustments necessary).

On day 1 (10/31/2006), pig 208 was found dead in the morning.

# TABLE D-19  ANIMAL HEALTH

**Naxcel Treatment**[a]

| First Day of Treatment | Pig | Group | Treatment Duration |
|---|---|---|---|
| Day 1 (10/31/06) | 233 | 7 | 3 Days |
| Day 3 (11/2/06) | 210 | 1 | 4 Days |
| | 229 | 2 | 11 Days |
| | 240 | 3 | 3 Days |
| Day 4 (11/3/06) | 221 | 2 | 3 Days |
| Day 6 (11/5/06) | 212 | 1 | 3 Days |
| Day 7 (11/6/06) | 232 | 2 | 7 Days |
| | 209 | 3 | 3 Days |
| | 235 | 4 | 3 Days |
| Day 10 (11/9/06) | 221 | 2 | 4 Days |

[a] Pigs were dosed with 1 mL/day Naxcel for diarhea, inappetance, fever, vomiting, and general illness.

**Other**
On day 1 (10/31/06), pig 208 (group 1) was found dead in the morning; the animal was necropsied and tissue samples were taken for pathology.  No significant pathological organisms were recovered and the necropsy was inconclusive, although the animal had a history of diarrhea.

# TABLE D-20  LATE DOSE CONSUMPTION

| Study Day | Pig | Dose* |
|-----------|-----|-------|
| Day 2 | 220 | PM |
|  | 223 | PM |
|  | 225 | PM |
|  | 240 | PM |
| Day 3 | 202 | AM |
|  | 220 | AM |
|  | 242 | AM |
| Day 4 | 209 | AM |
|  | 220 | AM |
| Day 5 | 202 | PM |
|  | 223 | PM |
| Day 6 | 221 | AM |
| Day 7 | 202 | AM |
|  | 220 | AM |
|  | 223 | PM |
| Day 8 | 202 | AM and PM |
|  | 209 | PM |
|  | 211 | PM |
|  | 223 | AM and PM |
| Day 9 | 202 | PM |
| Day 10 | 202 | PM |
|  | 223 | PM |

*Dose was consumed by feeding time, but not immediately; typically, dose was eaten over the hour following dosing.

See Table A-3 for missed doses.

## TABLE D-21  URINE VOLUMES

| Group | Pig Number | Urine Collection[a] | | |
| --- | --- | --- | --- | --- |
| | | U-1 Days 6-7 | U-2 Days 9-10 | U-3 Days 12-13 |
| 1 | 208 | no sample[b] | no sample[b] | no sample[b] |
| | 210 | 16830 | 29280 | 37500 |
| | 212 | 1280 | 4095 | 4370 |
| | 219 | 4320 | 4700 | 4360 |
| | 234 | 3170 | 4036 | 5190 |
| 2 | 201 | 12000 | 14810 | 16720 |
| | 205 | 11180 | 9500 | 15070 |
| | 221 | 6660 | 9300 | 12560 |
| | 229 | 2800 | 3540 | 5210 |
| | 232 | 4630 | 7160 | 5690 |
| 3 | 203 | 1910 | 1310 | 2830 |
| | 204 | 3620 | 3010 | 4580 |
| | 209 | 4096 | 4920 | 9800 |
| | 211 | 12640 | 12410 | 14200 |
| | 240 | 2470 | 3460 | 3800 |
| 4 | 214 | 4500 | 8430 | 11350 |
| | 224 | 5260 | 9000 | 11330 |
| | 231 | 41140 | 33400 | 32500 |
| | 235 | 5220 | 9560 | 9200 |
| | 242 | 6064 | 5250 | 9790 |
| 5 | 220 | 11290 | 14550 | 13890 |
| | 222 | 6690 | 8220 | 13130 |
| | 226 | 4840 | 4063 | 3190 |
| | 228 | 7080 | 9010 | 9520 |
| | 241 | 5640 | 6850 | 6840 |
| 6 | 202 | 5040 | 10540 | 8920 |
| | 207 | 16790 | 15740 | 17525 |
| | 217 | 3500 | 3740 | 5600 |
| | 223 | 7620 | 7360 | 6143 |
| | 225 | 4095 | 6720 | 3990 |
| 7 | 227 | 3040 | 5340 | 9690 |
| | 233 | 12580 | 13350 | 18000 |
| | 237 | 12740 | 8480 | 8163 |

Units = milliliters
[a] Urine was collected over 48-hour periods.
[b] Pig 208 died on day 1, before urine collections occurred.

# TABLE D-22  URINARY CHROMIUM ANALYTICAL RESULTS FOR STUDY SAMPLES

| Sample Number | Tag Number | Pig Number | Group | Material Administered | Urine Collection Days | 48-hr Dose (ug/48hr) | 48-hr BWAdj Dose (ug/kg-48hr) | Q | Reported Conc (ng/mL) | AdjConc*( ng/mL) | Urine Volume (mL) | Total Excreted (ug/48hrs) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Navy2Cr-208-U3 | Navy2Cr-204 | 208 | 1 | Chromium Chloride | 12/13 | No data -- pig was found dead on day 1. | | | | | | |
| Navy2Cr-210-U3 | Navy2Cr-211 | 210 | 1 | Chromium Chloride | 12/13 | 6600 | 479.22 | < | 3 | 1.5 | 37500 | 56 |
| Navy2Cr-212-U3 | Navy2Cr-213 | 212 | 1 | Chromium Chloride | 12/13 | 6600 | 495.38 | | 9.2 | 9.2 | 4370 | 40 |
| Navy2Cr-219-U3 | Navy2Cr-191 | 219 | 1 | Chromium Chloride | 12/13 | 6600 | 472.39 | | 4 | 4 | 4360 | 17 |
| Navy2Cr-234-U3 | Navy2Cr-197 | 234 | 1 | Chromium Chloride | 12/13 | 6600 | 499.16 | | 9 | 9 | 5190 | 47 |
| Navy2Cr-232-U3 | Navy2Cr-183 | 232 | 2 | Chromium Chloride | 12/13 | 13200 | 1056.37 | | 6 | 6 | 5690 | 34 |
| Navy2Cr-201-U3 | Navy2Cr-193 | 201 | 2 | Chromium Chloride | 12/13 | 13200 | 985.33 | < | 3 | 1.5 | 16720 | 25 |
| Navy2Cr-205-U3 | Navy2Cr-209 | 205 | 2 | Chromium Chloride | 12/13 | 13200 | 992.67 | < | 3 | 1.5 | 15070 | 23 |
| Navy2Cr-221-U3 | Navy2Cr-185 | 221 | 2 | Chromium Chloride | 12/13 | 13200 | 1023.54 | | 3 | 3 | 12560 | 38 |
| Navy2Cr-229-U3 | Navy2Cr-217 | 229 | 2 | Chromium Chloride | 12/13 | 13200 | 929.79 | | 5 | 5 | 5210 | 26 |
| Navy2Cr-240-U3 | Navy2Cr-194 | 240 | 3 | Chromium Chloride | 12/13 | 19600 | 1493.77 | | 12 | 12 | 3800 | 46 |
| Navy2Cr-211-U3 | Navy2Cr-202 | 211 | 3 | Chromium Chloride | 12/13 | 19600 | 1488.1 | | 8 | 8 | 14200 | 114 |
| Navy2Cr-209-U3 | Navy2Cr-205 | 209 | 3 | Chromium Chloride | 12/13 | 19600 | 1320.15 | | 8 | 8 | 9800 | 78 |
| Navy2Cr-204-U3 | Navy2Cr-179 | 204 | 3 | Chromium Chloride | 12/13 | 19600 | 1420.59 | | 16 | 16 | 4580 | 73 |
| Navy2Cr-203-U3 | Navy2Cr-212 | 203 | 3 | Chromium Chloride | 12/13 | 19600 | 1338.27 | | 15 | 15 | 2830 | 42 |
| Navy2Cr-231-U3 | Navy2Cr-215 | 231 | 4 | Test Material 2 | 12/13 | 10407.1 | 724.24 | < | 3 | 1.5 | 32500 | 49 |
| Navy2Cr-224-U3 | Navy2Cr-208 | 224 | 4 | Test Material 2 | 12/13 | 10407.1 | 683.63 | < | 3 | 1.5 | 11330 | 17 |
| Navy2Cr-214-U3 | Navy2Cr-192 | 214 | 4 | Test Material 2 | 12/13 | 10407.1 | 734 | | 5 | 5 | 11350 | 57 |
| Navy2Cr-242-U3 | Navy2Cr-186 | 242 | 4 | Test Material 2 | 12/13 | 10407.1 | 672.75 | | 3 | 3 | 9790 | 29 |
| Navy2Cr-235-U3 | Navy2Cr-203 | 235 | 4 | Test Material 2 | 12/13 | 10407.1 | 688.23 | | 4 | 4 | 9200 | 37 |
| Navy2Cr-226-U3 | Navy2Cr-216 | 226 | 5 | Test Material 2 | 12/13 | 16910.95 | 1191.08 | | 32 | 32 | 3190 | 102 |
| Navy2Cr-228-U3 | Navy2Cr-200 | 228 | 5 | Test Material 2 | 12/13 | 16699.56 | 1146.11 | | 4 | 4 | 9520 | 38 |
| Navy2Cr-220-U3 | Navy2Cr-189 | 220 | 5 | Test Material 2 | 12/13 | 16910.95 | 1186.77 | < | 3 | 1.5 | 13890 | 21 |
| Navy2Cr-222-U3 | Navy2Cr-214 | 222 | 5 | Test Material 2 | 12/13 | 16910.95 | 1245.95 | | 11 | 11 | 13130 | 144 |
| Navy2Cr-241-U3 | Navy2Cr-180 | 241 | 5 | Test Material 2 | 12/13 | 16910.95 | 1243.64 | | 3 | 3 | 6840 | 21 |
| Navy2Cr-225-U3 | Navy2Cr-199 | 225 | 6 | Test Material 2 | 12/13 | 23417.16 | 1781 | | 11 | 11 | 3990 | 44 |
| Navy2Cr-202-U3 | Navy2Cr-181 | 202 | 6 | Test Material 2 | 12/13 | 23417.16 | 1788.06 | | 5 | 5 | 8920 | 45 |
| Navy2Cr-207-U3 | Navy2Cr-195 | 207 | 6 | Test Material 2 | 12/13 | 23417.16 | 1744.54 | | 20 | 20 | 17525 | 351 |
| Navy2Cr-217-U3 | Navy2Cr-187 | 217 | 6 | Test Material 2 | 12/13 | 23417.16 | 1609.64 | | 6 | 6 | 5600 | 34 |
| Navy2Cr-223-U3 | Navy2Cr-210 | 223 | 6 | Test Material 2 | 12/13 | 23417.16 | 1604.17 | | 6 | 6 | 6143 | 37 |
| Navy2Cr-227-U3 | Navy2Cr-188 | 227 | 7 | Control | 12/13 | 0 | 0 | < | 3 | 1.5 | 9690 | 15 |
| Navy2Cr-233-U3 | Navy2Cr-184 | 233 | 7 | Control | 12/13 | 0 | 0 | < | 3 | 1.5 | 18000 | 27 |
| Navy2Cr-237-U3 | Navy2Cr-182 | 237 | 7 | Control | 12/13 | 0 | 0 | < | 3 | 1.5 | 8163 | 12 |
| Navy2Cr-210-U1 | Navy2Cr-131 | 210 | 1 | Chromium Chloride | 6/7 | 6600 | 569.11 | | 4 | 4 | 16830 | 67 |
| Navy2Cr-208-U1 | Navy2Cr-124 | 208 | 1 | Chromium Chloride | 6/7 | No data -- pig was found dead on day 1. | | | | | | |
| Navy2Cr-234-U1 | Navy2Cr-101 | 234 | 1 | Chromium Chloride | 6/7 | 6600 | 604.25 | | 12 | 12 | 3170 | 38 |
| Navy2Cr-212-U1 | Navy2Cr-130 | 212 | 1 | Chromium Chloride | 6/7 | 6600 | 575.67 | | 32 | 32 | 1280 | 41 |
| Navy2Cr-219-U1 | Navy2Cr-115 | 219 | 1 | Chromium Chloride | 6/7 | 6600 | 571.55 | | 6 | 6 | 4320 | 26 |
| Navy2Cr-232-U1 | Navy2Cr-120 | 232 | 2 | Chromium Chloride | 6/7 | 13200 | 1269.35 | | 18 | 18 | 4630 | 83 |
| Navy2Cr-229-U1 | Navy2Cr-133 | 229 | 2 | Chromium Chloride | 6/7 | 13200 | 1069.06 | | 13 | 13 | 2800 | 36 |
| Navy2Cr-221-U1 | Navy2Cr-116 | 221 | 2 | Chromium Chloride | 6/7 | 11550 | 1089.92 | | 9.6 | 9.6 | 6660 | 64 |
| Navy2Cr-201-U1 | Navy2Cr-126 | 201 | 2 | Chromium Chloride | 6/7 | 13200 | 1189.51 | | 6 | 6 | 12000 | 72 |
| Navy2Cr-205-U1 | Navy2Cr-137 | 205 | 2 | Chromium Chloride | 6/7 | 13200 | 1194.9 | | 3 | 3 | 11180 | 34 |
| Navy2Cr-211-U1 | Navy2Cr-112 | 211 | 3 | Chromium Chloride | 6/7 | 19600 | 1802.48 | | 3 | 3 | 12640 | 38 |
| Navy2Cr-203-U1 | Navy2Cr-129 | 203 | 3 | Chromium Chloride | 6/7 | 19600 | 1647.45 | | 3.7 | 3.7 | 1910 | 7 |
| Navy2Cr-240-U1 | Navy2Cr-122 | 240 | 3 | Chromium Chloride | 6/7 | 19600 | 1810.93 | | 30 | 30 | 2470 | 74 |
| Navy2Cr-209-U1 | Navy2Cr-109 | 209 | 3 | Chromium Chloride | 6/7 | 19600 | 1647.32 | | 27 | 27 | 4096 | 111 |
| Navy2Cr-204-U1 | Navy2Cr-118 | 204 | 3 | Chromium Chloride | 6/7 | 19600 | 1704.71 | | 21 | 21 | 3620 | 76 |
| Navy2Cr-231-U1 | Navy2Cr-128 | 231 | 4 | Test Material 2 | 6/7 | 10407.1 | 895.57 | | 4 | 4 | 41140 | 165 |
| Navy2Cr-224-U1 | Navy2Cr-104 | 224 | 4 | Test Material 2 | 6/7 | 10407.1 | 829.43 | | 4 | 4 | 5260 | 21 |
| Navy2Cr-242-U1 | Navy2Cr-113 | 242 | 4 | Test Material 2 | 6/7 | 10407.1 | 826.17 | | 3 | 3 | 6064 | 18 |
| Navy2Cr-235-U1 | Navy2Cr-127 | 235 | 4 | Test Material 2 | 6/7 | 10407.1 | 842.9 | | 7 | 7 | 5220 | 37 |
| Navy2Cr-214-U1 | Navy2Cr-135 | 214 | 4 | Test Material 2 | 6/7 | 10407.1 | 811.59 | | 9 | 9 | 4500 | 41 |
| Navy2Cr-228-U1 | Navy2Cr-102 | 228 | 5 | Test Material 2 | 6/7 | 16910.95 | 1378.01 | < | 3 | 1.5 | 7080 | 11 |
| Navy2Cr-241-U1 | Navy2Cr-111 | 241 | 5 | Test Material 2 | 6/7 | 16699.56 | 1465.29 | | 6 | 6 | 5640 | 34 |
| Navy2Cr-226-U1 | Navy2Cr-139 | 226 | 5 | Test Material 2 | 6/7 | 16910.95 | 1445.62 | | 14 | 14 | 4840 | 68 |
| Navy2Cr-220-U1 | Navy2Cr-132 | 220 | 5 | Test Material 2 | 6/7 | 14797.08 | 1238.13 | | 5 | 5 | 11290 | 56 |
| Navy2Cr-222-U1 | Navy2Cr-107 | 222 | 5 | Test Material 2 | 6/7 | 16910.95 | 1470.96 | | 5 | 5 | 6690 | 33 |
| Navy2Cr-223-U1 | Navy2Cr-136 | 223 | 6 | Test Material 2 | 6/7 | 23417.16 | 1912.03 | | 9.9 | 9.9 | 7620 | 75 |
| Navy2Cr-202-U1 | Navy2Cr-123 | 202 | 6 | Test Material 2 | 6/7 | 21368.16 | 1933.21 | | 47 | 47 | 5040 | 237 |
| Navy2Cr-207-U1 | Navy2Cr-105 | 207 | 6 | Test Material 2 | 6/7 | 23417.16 | 2036.55 | | 24 | 24 | 16790 | 403 |
| Navy2Cr-217-U1 | Navy2Cr-119 | 217 | 6 | Test Material 2 | 6/7 | 23417.16 | 1884.99 | | 39 | 39 | 3500 | 137 |
| Navy2Cr-225-U1 | Navy2Cr-103 | 225 | 6 | Test Material 2 | 6/7 | 23417.16 | 2110.04 | | 6 | 6 | 4095 | 25 |
| Navy2Cr-237-U1 | Navy2Cr-134 | 237 | 7 | Control | 6/7 | 0 | 0 | < | 3 | 1.5 | 12740 | 19 |
| Navy2Cr-233-U1 | Navy2Cr-106 | 233 | 7 | Control | 6/7 | 0 | 0 | < | 3 | 1.5 | 12580 | 19 |
| Navy2Cr-227-U1 | Navy2Cr-108 | 227 | 7 | Control | 6/7 | 0 | 0 | < | 3 | 1.5 | 3040 | 5 |
| Navy2Cr-234-U2 | Navy2Cr-166 | 234 | 1 | Chromium Chloride | 9/10 | 6600 | 549.02 | | 11 | 11 | 4036 | 44 |
| Navy2Cr-208-U2 | Navy2Cr-163 | 208 | 1 | Chromium Chloride | 9/10 | No data -- pig was found dead on day 1. | | | | | | |
| Navy2Cr-210-U2 | Navy2Cr-164 | 210 | 1 | Chromium Chloride | 9/10 | 6600 | 520.81 | < | 3 | 1.5 | 29280 | 44 |

| Sample Number | Tag Number | Pig Number | Group | Material Administered | Urine Collection Days | 48-hr Dose (ug/48hr) | 48-hr BWAdj Dose (ug/kg-48hr) | Q | Reported Conc (ng/mL) | AdjConc*( ng/mL) | Urine Volume (mL) | Total Excreted (ug/48hrs) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Navy2Cr-212-U2 | Navy2Cr-157 | 212 | 1 | Chromium Chloride | 9/10 | 6600 | 520.72 | | 11 | 11 | 4095 | 45 |
| Navy2Cr-219-U2 | Navy2Cr-171 | 219 | 1 | Chromium Chloride | 9/10 | 6600 | 520.85 | | 3 | 3 | 4700 | 14 |
| Navy2Cr-205-U2 | Navy2Cr-150 | 205 | 2 | Chromium Chloride | 9/10 | 13200 | 1084.46 | | 3 | 3 | 9500 | 29 |
| Navy2Cr-221-U2 | Navy2Cr-161 | 221 | 2 | Chromium Chloride | 9/10 | 13200 | 1130.93 | | 7 | 7 | 9300 | 65 |
| Navy2Cr-229-U2 | Navy2Cr-168 | 229 | 2 | Chromium Chloride | 9/10 | 13200 | 998.18 | | 12 | 12 | 3540 | 42 |
| Navy2Cr-232-U2 | Navy2Cr-175 | 232 | 2 | Chromium Chloride | 9/10 | 13200 | 1173.65 | | 5 | 5 | 7160 | 36 |
| Navy2Cr-201-U2 | Navy2Cr-141 | 201 | 2 | Chromium Chloride | 9/10 | 13200 | 1082.21 | < | 3 | 1.5 | 14810 | 22 |
| Navy2Cr-211-U2 | Navy2Cr-147 | 211 | 3 | Chromium Chloride | 9/10 | 18620 | 1571.83 | | 9.5 | 9.5 | 12410 | 118 |
| Navy2Cr-209-U2 | Navy2Cr-145 | 209 | 3 | Chromium Chloride | 9/10 | 19600 | 1477.26 | | 9 | 9 | 4920 | 44 |
| Navy2Cr-240-U2 | Navy2Cr-154 | 240 | 3 | Chromium Chloride | 9/10 | 19600 | 1654.48 | | 18 | 18 | 3460 | 62 |
| Navy2Cr-204-U2 | Navy2Cr-152 | 204 | 3 | Chromium Chloride | 9/10 | 19600 | 1555.95 | | 20 | 20 | 3010 | 60 |
| Navy2Cr-203-U2 | Navy2Cr-165 | 203 | 3 | Chromium Chloride | 9/10 | 19600 | 1488.17 | | 38 | 38 | 1310 | 50 |
| Navy2Cr-214-U2 | Navy2Cr-151 | 214 | 4 | Test Material 2 | 9/10 | 10407.1 | 805.27 | | 3 | 3 | 8430 | 25 |
| Navy2Cr-224-U2 | Navy2Cr-174 | 224 | 4 | Test Material 2 | 9/10 | 10407.1 | 747.66 | < | 3 | 1.5 | 9000 | 14 |
| Navy2Cr-231-U2 | Navy2Cr-149 | 231 | 4 | Test Material 2 | 9/10 | 10407.1 | 805.4 | < | 3 | 1.5 | 33400 | 50 |
| Navy2Cr-235-U2 | Navy2Cr-146 | 235 | 4 | Test Material 2 | 9/10 | 10407.1 | 759.9 | < | 3 | 1.5 | 9560 | 14 |
| Navy2Cr-242-U2 | Navy2Cr-178 | 242 | 4 | Test Material 2 | 9/10 | 10407.1 | 748.92 | | 4 | 4 | 5250 | 21 |
| Navy2Cr-226-U2 | Navy2Cr-173 | 226 | 5 | Test Material 2 | 9/10 | 16910.95 | 1308.91 | | 20 | 20 | 4063 | 81 |
| Navy2Cr-228-U2 | Navy2Cr-170 | 228 | 5 | Test Material 2 | 9/10 | 16910.95 | 1257.6 | < | 3 | 1.5 | 9010 | 14 |
| Navy2Cr-222-U2 | Navy2Cr-172 | 222 | 5 | Test Material 2 | 9/10 | 16910.95 | 1345.02 | | 9.5 | 9.5 | 8220 | 78 |
| Navy2Cr-220-U2 | Navy2Cr-169 | 220 | 5 | Test Material 2 | 9/10 | 16910.95 | 1274.32 | < | 3 | 1.5 | 14550 | 22 |
| Navy2Cr-241-U2 | Navy2Cr-176 | 241 | 5 | Test Material 2 | 9/10 | 16910.95 | 1358.72 | < | 3 | 1.5 | 6850 | 10 |
| Navy2Cr-202-U2 | Navy2Cr-167 | 202 | 6 | Test Material 2 | 9/10 | 23417.16 | 1968.3 | | 11 | 11 | 10540 | 116 |
| Navy2Cr-207-U2 | Navy2Cr-177 | 207 | 6 | Test Material 2 | 9/10 | 23417.16 | 1885.06 | | 18 | 18 | 15740 | 283 |
| Navy2Cr-217-U2 | Navy2Cr-160 | 217 | 6 | Test Material 2 | 9/10 | 23417.16 | 1738.17 | | 11 | 11 | 3740 | 41 |
| Navy2Cr-223-U2 | Navy2Cr-142 | 223 | 6 | Test Material 2 | 9/10 | 23417.16 | 1744.72 | | 11 | 11 | 7360 | 81 |
| Navy2Cr-225-U2 | Navy2Cr-155 | 225 | 6 | Test Material 2 | 9/10 | 23417.16 | 1931.8 | | 4 | 4 | 6720 | 27 |
| Navy2Cr-227-U2 | Navy2Cr-143 | 227 | 7 | Control | 9/10 | 0 | 0 | < | 3 | 1.5 | 5340 | 8 |
| Navy2Cr-233-U2 | Navy2Cr-158 | 233 | 7 | Control | 9/10 | 0 | 0 | < | 3 | 1.5 | 13350 | 20 |
| Navy2Cr-237-U2 | Navy2Cr-162 | 237 | 7 | Control | 9/10 | 0 | 0 | < | 3 | 1.5 | 8480 | 13 |

Q = Data qualifier
*Non-detects taken at one-half the detection limit.

# TABLE D-23  CHROMIUM ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

**Blind Duplicates**

| Tag Number | Group | Event | DL | Duplicate Conc | Original Pig # |
|---|---|---|---|---|---|
| Navy2Cr-117 | 2 | U1 | 3 | 5 | 201 |
| Navy2Cr-121 | 6 | U1 | 3 | 23 | 207 |
| Navy2Cr-138 | 7 | U1 | 3 | <3 | 237 |
| Navy2Cr-148 | 3 | U2 | 3 | 21 | 204 |
| Navy2Cr-140 | 5 | U2 | 3 | 8 | 222 |
| Navy2Cr-190 | 2 | U3 | 3 | <3 | 221 |
| Navy2Cr-201 | 4 | U3 | 3 | 7 | 214 |
| Navy2Cr-207 | 6 | U3 | 3 | 9.4 | 225 |

**PE Sample Analysis**

| Tag Number | QC Sample | Nominal Conc | Measured Conc | DL |
|---|---|---|---|---|
| Navy2Cr-110 | Chromium (III) chloride | 50 | 250 | 3 |
| Navy2Cr-159 | Chromium (III) chloride | 50 | 250 | 3 |
| Navy2Cr-206 | Chromium (III) chloride | 50 | 270 | 3 |
| Navy2Cr-125 | Chromium (III) chloride | 100 | 520 | 6 |
| Navy2Cr-153 | Chromium (III) chloride | 100 | 460 | 6 |
| Navy2Cr-196 | Chromium (III) chloride | 100 | 500 | 6 |
| Navy2Cr-114 | Chromium (III) chloride | 200 | 1100 | 20 |
| Navy2Cr-156 | Chromium (III) chloride | 200 | 1100 | 20 |
| Navy2Cr-198 | Chromium (III) chloride | 200 | 1200 | 20 |

**Laboratory Control Standards**

| Reference Material | Certified Mean | DL | Measured Conc |
|---|---|---|---|
| NIST 1640 | 38.6 ± 1.6 | 1 | 36 |
| NIST 1640 | 38.6 ± 1.6 | 1 | 34 |
| NIST 1640 | 38.6 ± 1.6 | 1 | 37 |
| Fluka-40-Cr | 40 ± 4 | 1 | 40 |
| Fluka-40-Cr | 40 ± 4 | 1 | 35 |
| Fluka-40-Cr | 40 ± 4 | 1 | 43 |

**Duplicates**

| Tag Number | DL | Duplicate Result | Original Sample Conc |
|---|---|---|---|
| Navy2Cr-103 | 3 | 6 | 6 |
| Navy2Cr-112 | 3 | 3 | 3 |
| Navy2Cr-132 | 3 | 6 | 5 |
| Navy2Cr-141 | 3 | <3 | <3 |
| Navy2Cr-150 | 3 | <3 | 3 |
| Navy2Cr-161 | 3 | 7 | 7 |
| Navy2Cr-170 | 3 | <3 | <3 |
| Navy2Cr-179 | 3 | 15 | 16 |
| Navy2Cr-188 | 3 | <3 | <3 |
| Navy2Cr-199 | 3 | 9 | 11 |
| Navy2Cr-209 | 3 | 3 | <3 |

**Blanks**

| Tag Number | DL | Measured Conc |
|---|---|---|
| Blank-1 | 3 | <3 |
| Blank-2 | 3 | <3 |
| Blank-3 | 3 | <3 |
| Blank-4 | 3 | <3 |
| Blank-5 | 3 | <3 |
| Blank-6 | 3 | <3 |

**Spikes (nominal spike: 300 ng/mL)**

| Tag Number | DL | Original Sample Conc | Spiked Result |
|---|---|---|---|
| Navy2Cr-108 | 3 | <3 | 320 |
| Navy2Cr-116 | 3 | 9.6 | 260 |
| Navy2Cr-128 | 3 | 4 | 330 |
| Navy2Cr-136 | 3 | 9.9 | 270 |
| Navy2Cr-146 | 3 | <3 | 340 |
| Navy2Cr-155 | 3 | 4 | 260 |
| Navy2Cr-166 | 3 | 11 | 310 |
| Navy2Cr-174 | 3 | <3 | 260 |
| Navy2Cr-184 | 3 | <3 | 260 |
| Navy2Cr-193 | 3 | <3 | 270 |
| Navy2Cr-214 | 3 | 11 | 260 |

DL = Detection limit
Units:  ng/mL

# APPENDIX E

# ABILITY OF SOIL PROPERTIES AND IN VITRO GASTROINTESTINAL EXTRACTIONS TO PREDICT BIOAVAILABILITY AND BIOACCESSIBILITY OF AS, PB, AND /OR CR IN SOIL

## ABILITY OF SOIL PROPERTIES TO PREDICT CONTAMINANT BIOAVAILABILITY IN ESTCP SOILS

Key soil physical and chemical properties (e.g. particle size, CEC, Fe-oxides, TOC/TIC, pH) were identified as controlling the extent of toxic metals bioaccessibility as measured using an in-vitro Physiologically-Based Extraction Test (PBET) that simulated the digestive system of humans. Statistical models were developed and incorporated into a predictive tool known as Soil BioAccessibility Tool (SBAT). The bioaccessibility results (in-vitro) were found to be in excellent agreement with molecular-level metal speciation studies and in-vivo swine metal bioavailability studies, which confirmed that key soil properties control metal bioavailability.

**Prediction of in vivo RBA from in vitro bioaccessibility**

The main objective of the project was to determine the ability of in vitro gastrointestinal methods (i.e., bioaccessibility methods) to predict measured contaminant bioavailability in contaminated soils from study sites. Equations used to predict bioavailability from bioaccessibility methods are available for Pb and As. Results are summarized and discussed in the following sections.

Lead

Drexler and Brattin (2007) reported the following prediction equations to calculate relative bioavailability of Pb from their relative bioaccessibility leaching procedure (RBALP). The RBALP method is the same as the PBET method used in this study.

RBA Pb (%) = 1.1368 IVBA (pH 1.5) – 7.79        $r^2 = 0.8241$

RBA Pb (%) = 1.3409 IVBA (pH 2.5) – 1.607        $r^2 = 0.7531$


Relative bioavailable Pb was determined for the Portsmouth soil in our study . Comparison of measured and predicted RBA Pb for the Portsmouth soil are shown in Table E-1.

**Table E-1.** Comparison of measured and predicted RBA Pb for the Portsmouth soil

| Measured Pb RBA, % | | Predicted Pb RBA | | | | |
|---|---|---|---|---|---|---|
| | | PBET pH 1.5 | | PBET pH 2.5 | | OSU IVG pH 1.8 |
| Mean | 90 % CI† | IVBA, % | RBA, % | IVBA, % | RBA, % | IVBA, % |
| 99 | 70 - 127 | 83.3 | 86.9 | 80.4 | 106.2 | 102.5 |

† CI = confidence interval

The PBET methods (pH 1.5 and 2.5) were able to accurately predict in vivo RBA for the Portsmouth soil.   The predicted RBA for the PBET method at pH 2.5 was closer to actual in vivo RBA than pH 1.5.  However both methods predict RBA Pb within the 90% C.I.   The OSU IVG method IVBA Pb was very close to the in vivo RBA Pb.  However, information on the ability of the OSU IVG method to predict RBA Pb is very limited whereas in depth validation studies have been conducted for the RBALP (i.e., PBET) method.

These results support the PBET methods of pH 1.5 and 2.5 can accurately predict in vivo RBA Pb.   Although the study was limited to determination of in vivo Pb RBA in soil, results support use of the PBET method. Future validation studies where this approach is expanded from the Portsmouth soil will increase the confidence of using in vitro methods to predict in vivo RBA Pb.   Results suggest that similar "PBET" in vitro methods (i.e., OSU IVG) may also be able to estimate RBA Pb in contaminated soils.

Arsenic

Several studies have reported correlation between bioaccessible As and bioavailable As measured from juvenile swine dosing trials.  These include the OSU IVG method (Basta et al., 2007; Rodriguez et al., 1999) and the SBET method (Juhasz et al. 2009).  The OSU IVG method reported by Rodriguez et al. (1999) incorporated the dosing vehicle used in the swine dosing trial study into its in vitro solution for 14 As contaminated soils.  Basta et al. (2007) reported results from the OSU IVG method with and without dosing vehicle for a subset of 9 soils used in Rodriguez et al. (1999).  In this study, the following regression equations used to predict RBA As were determined from the OSU IVG procedure without dosing vehicle (Basta et al., 2007) for the 14 soils used in Rodriguez et al. (1999).

Bioaccessible As measured under gastric conditions:
RBA As (%) = 0.906 IVBA As + 7.37        $r^2$ =0.85

Bioaccessible As measured under intestinal conditions:
RBA As (%) = 1.02 IVBA As + 7.55         $r^2$ =0.82

The SBRC method of Juhasz et. al (2009) is identical to the PBET pH of 1.5 method used in our study.  The following regression from the SBRC method (Juhasz et al., 2009) was used to predict the RBA As for the Deseret soil using PBET pH 1.5 IVBA As data.

RBA As (%) = 1.656 IVBA As + 0.992        $r^2$ =0.75

Comparison of measured and predicted RBA As for the Deseret soil are shown in Table E-2.

**Table E-2.** Comparison of measured and predicted RBA As for the Deseret soil

| Measured As RBA, % | | Predicted As RBA | | | | | |
|---|---|---|---|---|---|---|---|
| | | OSU IVG gastric | | OSU IVG intestinal | | SBET gastric | |
| Mean | 90 % CI† | IVBA, % | RBA, % | IVBA, % | RBA, % | IVBA, % | RBA, % |
| 14 | 13-15 | 8.45 | 15.0 | 8.47 | 16.2 | 10.6 | 12.2 |

† CI = confidence interval

In general, all of the in vitro methods predicted in vivo RBA As with 90% confidence.

These results support that the OSU IVG methods (gastric and intestinal) and the SBRC method (PBET, pH 1.5) can accurately predict in vivo RBA As. However, the study was limited to determination of in vivo As RBA in one soil. The source of As contamination in the Desert soil was associated with previous mining activities. Soils contaminated with As from mining activities were used to derive the RBA As prediction equations for the OSU IVG method.

The effect of arsenic contaminant speciation on the ability of in vitro methods to predict RBA As in not known. The contaminant chemical speciation of arsenic of the mining soils used in the OSU IVG is likely to be more similar to speciation in the mining contaminated Desert soil than other contaminant sources (i.e., arsenical pesticides). Therefore application of an in vivo RBA As prediction equation from in vitro bioaccessible As developed using mining soils may be more accurate for the Desert soil than using prediction equations developed using different contaminant sources.

The 12 As contamination sources used to derive the RBA As prediction equations for the SBRC method were more diverse. The number of soils, in parentheses, from contaminant sources reported in Juhasz et al. (2009) were: railway corridors (6), dip sites (2), mine sites (2), and gossans (2). However, the SBRC method provided an accurate prediction of RBA As in the Deseret soil. Juhasz et al. (2009) reported RBA As prediction equations derived from the OSU IVG method for the 12 soils in their study.

Future validation studies where this approach is expanded from the Deseret soil to other soils contaminated with arsenic will increase the confidence of using in vitro methods to predict in vivo RBA As. Predicted RBA As by the OSU IVG method determined using Juhasz et al. (2009) is 21.5% for gastric phase and 23.3% for the intestinal phase. These results suggest OSU IVG would overpredict bioavailable As by 6.5% (gastric) or 8.3% (intestinal). Overprediction may be viewed as a desirable conservative measure by regulators. However, the correlation between the OSU IVG method and RBA As reported by Juhasz et al. (2009) of r2=0.57 (gastric and intestinal) was much weaker than reported by Basta et al. (2003) of r2=0.85 (gastric) and r2=(0.81). Therefore, the regression with the higher prediction power was used to predict RBA As for our study soil. It is possible that the weaker correlation for the OSU IVG method reported by Juhasz et al. (2009) was due to different contaminant speciation in a few of the non-mining soils. Further research on the effect of contaminant speciation of different contaminant sources on the ability of various in vitro methods to predict RBA As is needed and is underway.

Results from our study show both the OSU IVG and SBRC method was able to predict RBA As in the Desert soil. The predicted RBA As by all methods ranged from 12.2 % to 16.2% which is comparable to the in vivo RBA As 14%. Further validation studies of these methods for other contaminated soils from different contaminant sources are warranted.


Chromium
A study investigating the relationship between in vitro IVBA Cr and in vivo RBA Cr has not been reported. Thus, it was not possible to evaluate the ability of bioaccessible Cr to predict in vivo RBA Cr. In our study, a novel immature swine dosing model was used to determine the in vivo RBA Cr for the McClellan soil. RBA Cr was 107% with a 90% confidence interval ranging

from 76% to 169%. In vitro IVBA Cr PBET method, used to measure bioaccessible Cr at pH 1.5 and at pH 2.5, was 10.1% and 19.0%, respectively. The in vitro IVBA values were much lower than the in vivo RBA Cr. Further research is needed before IVBA can be used to predict in vivo RBA Cr.

**Prediction of in vitro bioaccessibility using soil properties**

Study of the determination of soil properties on in vivo bioavailability or in vitro bioaccessibility is very limited. To our knowledge, these relationships has not been reported for Pb and limited studies exist for As and Cr. The ability of soil properties to predict bioaccessible As and Cr is discussed below.

<u>Arsenic</u>

Yang et al. (2002) reported the following relationship between in vitro bioaccessible As (IVBA As) and soil properties.

IVBA As (%) = 11.3 pH – 30.5 log Fe

Where pH is the soil pH and Fe is soil Fe extracted with citrate-bicarbonate-dithionite solution (CBD) in g/kg (Mehra and Jackson, 1960). Using the pH reported by Rodriguez et al. (1999) and CBD extractable Fe measured on these samples, the equation derived to predict IVBA As was able to predict RBA As within a root square mean error of 9.5%.

Similarly, Whitacre (2009) reported Fe oxide content and pH in 19 soils were able to predict IVBA As measured by the OSU IVG gastric and Intestinal phases. The following prediction equations were reported:

gastric conditions:
IVBA As (%) = 30.7 – 22.6 log Feox $\quad$ + 12.3 pH $\quad$ $R^2$ = 0.84
IVBA As (%) = 88.2 - 33.2 log FeCBD + 12.2 pH $\quad$ $R^2$ = 0.92

Intestinal conditions:
IVBA As (%) = 36.4 – 23.4 log Feox $\quad$ + 12.0 pH $\quad$ $R^2$ = 0.83
IVBA As (%) = 95.7 -34.3 log FeCBD $\quad$ + 11.9 pH $\quad$ $R^2$ = 0.92

where Feox is soil Fe determined by extraction with acid ammonium oxalate solution (in mg/kg) and FeCBD is soil Fe extracted with citrate bicarbonate dithionite (in mg/kg).

Use of above regression equations allow prediction of IVBA from soil properties. Comparison of measured and calculated IVBA As using soil property predictive models of Yang et al. (2002) and Whitacre (2008) for As contaminated study soils are summarized in Table E-3.

**Table E-3**. Comparison of measured and predicted IVBA As (%) for study soils with As contamination.

| Soil | Gastric | | | | | Gastrointestinal | | |
|------|------|------|------|------|------|------|------|------|
|  | M1 | P1 | P2 | M2 | P1 | M3 | P4 | P5 |
| Concord | 18.6 | 30.8 | 33.3 | 22.1 | 41.7 | 18.4 | 31.6 | 34.3 |
| Deseret | 8.45 | 76.7 | 75.9 | 10.6 | 81.0 | 8.47 | 77.2 | 76.4 |
| Hilo | 11.8 | -0.1 | 9.8 | 15.1 | 20.0 | 11.1 | 0.3 | 10.6 |
| PC | 14.6 | 13.5 | 27.5 | 43.3 | 36.4 | 13.8 | 13.3 | 27.9 |

M1 = measured IVBA As using OSU IVG
M2 = measured IVBA using PBET, pH 1.5
M3 = measured IVBA using OSU IVG
P1 = predicted using IVBA As (%) = 30.7 – 22.6 log Feox  + 12.3 pH (Whitacre, 2008)
P2 = predicted using IVBA As (%) = 88.2 - 33.2 log FeCBD + 12.2 pH (Whitacre, 2008)
P3 = predicted using IVBA As (%) = 11.3 pH – 30.5 log Fe (Yang et al. (2002)
P4 = predicted using IVBA As (%) = 36.4 – 23.4 log Feox  + 12.0 pH (Whitacre, 2008)
P5 = predicted using IVBA As (%) = 95.7 -34.3 log FeCBD  + 11.9 pH (Whitacre, 2008)

The predicted IVBA As by Whitacre using the OSU IVG method using CBD Fe were very close to the IVBA predicted from the equation reported by Yang et al. (2002) using the PBET pH 1.5 method. In general, predicted IVBA were similar between predictive equations using Feox or CBD Fe.

Soil properties of the Deseret soil were not predictive of the measured IVBA As. However, predicted and measured IVBA for the Concord, Hilo and PC were in good agreement. Soils with a wide range of properties were spiked with As in Yang et al. (2002) and in Whitacre (2009). Soluble As in the spike solution reacted with soil clays and other components that sorb the arsenic. Arsenical pesticide was the contaminant source in the Concord, Hilo and PC soils. Solid phase arsenic speciation (Table E-4) showed As was associated with Fe and possibly Al oxides for these 3 soils. Soluble arsenical pesticide likely reacted (i.e., sorbed, precipitated) to soil reactive components similar to soluble As spike solution.

However, the arsenic source was mining waste in the Deseret soil. Unlike the previous 3 soils, solid phase arsenic speciation (Table E-4) showed As associated with Fe was highly variable. This finding suggests arsenic may occur as discrete minerals from the mining operation. It is likely the insoluble As minerals in the mining waste did not appreciably dissolve and react with soil components. Therefore, its chemical speciation and IVBA solubility will depend on the mining waste mineral not soil property.

It is very possible that the poor prediction of soil properties to predict IVBA As in the Deseret soil may be due to different arsenic speciation in this mining contaminant soil than the spiked soils. Further research on the effect of contaminant speciation of different contaminant sources on the ability of various in vitro methods to predict RBA As is needed and is underway. Further validation studies of these methods for other contaminated soils from different contaminant sources are warranted.

The root square mean error (RSME) was used to evaluate the ability of each soil property driven model to predict its respective IVBA (Table E-5) .

**Table E-4.** Metal(loid) contaminant speciation of Study Soils

| Site Name | Site Location | Suspected contaminant source(s) | Metal-(loid) | Contaminant Speciation |
|---|---|---|---|---|
| MCAS Cherry Point | Cherry Point, NC | incinerator | Cr<br><br>Pb | Not chromite; associated with Mn, Bi<br><br>Non-crystalline β-PbO |
| Concord Naval Weapons | Concord, CA | pesticide use | As | Assoc. with Fe, Mn oxides<br>Not consistent with discrete mineral phases (e.g., scorodite, schultenite, arseniosiderite) |
| Deseret Chemical Depot | Tooele, UT | alluvial mine tailings | As | Highly variable assoc. with Fe |
| Hill AFB | Ogden, UT | Water treatment sludge drying bed | As<br>Cd<br>Cr | Nothing reported? |
| Sugar Cane Farm | Hilo, HI | pesticide use | As | Assoc. with Fe and Al oxides |
| McClellan AFB | Sacramento, CA | Wastewater treatment lagoon | Cr<br><br>Pb | Not chromite; heterogeneous distribution<br><br>associated with Mn, Fe and organic matter |
| NSA | Mechanicsburg, PA | lead ingot storage area | Pb | Non-crystalline β-PbO; similar to Travis<br>Elemental Pb<br>Not associated with Fe, Mn oxides |
| ORNL Firing Range | Oak Ridge, TN | small arms fire | Pb | Adsorbed to Fe, Mn oxides<br>Similar to McClellan |
| Pearl Harbor Fuel Depot | Pearl City, HI | pesticides | As<br><br>Pb | Both As(III) and As(V), predominantely As(III); Arsenite sorbed to ferrihyrite |
| Portsmouth Naval Shipyard | Kittery, ME | Lead battery cells | Pb | Inconsistent assoc. with Fe |
| Travis AFB | Fairfield, CA | small arms fire | Pb | Associated with Fe, Cr, Mn<br>Poorly crystalline Pb oxides |

Table E-5.  Comparison of root square mean errors (RSME) for predictive soil property models.

| | RSME, % | | | | |
| | Gastric | | | Gastrointestinal | |
| | PBET | OSU IVG | | OSU IVG | |
| | CBD Fe | Feox | CBD Fe | Feox | CBD Fe |
| All soils | 36.8 | 35.2 | 35.1 | 35.4 | 44.5 |
| All soils excluding Deseret | 3.50 | 3.42 | 3.42 | 3.44 | 3.85 |

RSME > 25% showed poor agreement between measured and predicted values for all prediction models.   However, very low RSME values of less than 4% showed excellent agreement between measured and predicted values was found for all models when the Deseret soil was excluded from the statistical analysis.   This clearly shows the ability of soil properties to predictive IVBA As is contaminant source dependent.  Good prediction was achieved for the non-mining arsenic source Concord, Hilo, and PC soils.   However, soil property models were not able to predict IVBA As in the mining arsenic source Deseret soil.

**Chromium**

Stewart et al. (2003) reported the following relationships between in vitro  bioaccessible Cr (IVBA Cr) and soil properties.

IVBA Cr (%) = 16.02 + (0.426 x %clay) - (9.56 x % TIC)

IVBA Cr (%) = 15.54 + (0.4908 x % clay) - (3.78 x %TOC)

where TIC is the soil inorganic carbon content and TOC is the soil organic C content.

 Use of above regression equations allow prediction of  IVBA from soil properties.  Comparison of measured  and calculated IVBA Cr using soil property predictive models of Stewart et al. (2003) are summarized in Table E-6.

**Table E-6.** Comparison of measured and predicted IVBA Cr (%) for study soils with Cr contamination.

| | IVBA Cr, % | | |
|---|---|---|---|
| Soil | M1 | P1 | P2 |
| Cherry Pt | 24.7 | 2.0 | -3.6 |
| Hill | 9.8 | 10.7 | 16.0 |
| McClellan | 10.1 | 26.3 | 10.1 |

M1 = measured IVBA using PBET, pH 1.5
P1 = predicted using IVBA Cr (%) = 16.02 + (0.426 x %clay) - (9.56 x % TIC)
P2 = predicted using IVBA Cr (%) = 15.54 + (0.4908 x % clay) - (3.78 x %TOC)

Good agreement between the measured IVBA Cr and predicted IVBA Cr by the P2 model were found for Hill and McClellan soils.  Poor agreement between the measured IVBA Cr and IVBA Cr predicted by P1 and P2 models was found for the Cherry Point soil.  Differences in Cr chemical speciation in soil may offer an explanation.  Water or wastewater treatment was the contaminant source for the Hill and McClellan soils.  Incinerator ash was the contaminant source for the Cherry Point soil.

The root square mean error (RSME) was used to evaluate the ability of each soil property driven model to predict its respective IVBA (Table E-7).

**Table E-7.** Comparison of root square mean errors (RSME) for IVBA Cr predictive soil property models.

| | RSME (%) for prediction models | |
|---|---|---|
| Soils | P1 | P2 |
| all | 16.5 | 12.2 |
| Hill and McClellan | 17.6 | 7.9 |

RSME values of less than 20% showed agreement between measured and predicted values was found for all models.  The RSME reduced to <10% for the P2 model when the Cherry Point soil was excluded from the statistical analysis.   This suggests the ability of soil properties to predictive IVBA Cr is contaminant source dependent.

# APPENDIX F

## Soil Properties and Metal(loid) Contaminant Concentrations

Soils from 12 study sites were collected.   Contaminated and allegedly uncontaminated reference soil was collected at each study site (Table F-1).   Soil samples from both contaminated and reference materials, ranging from 60 kg to 240 kg, were sent to The Ohio State University for processing and homogenization (see SOP in Materials and Methods).   Two size fractions, < 250 um and < 2 mm, of each soil were prepared at OSU and shipped to research team members.  The < 2 mm size fraction, defined as whole soil, was used for ecological plant and earthworm bioassay studies and < 250 um size fraction was used for human risk in vivo swine dosing and in vitro gastrointestinal extraction studies.  Soil properties, important to plant and earthworm bioassays and contaminant bioavailability, were determined for both < 2 mm and < 250 um fractions. Elemental content of major soil constituents and/or soil metal(loid) contaminants were determined for both soil size fractions.  Total contaminant concentration in < 2 mm soil (Table F-2) and < 250 um  (Table F-3) are summarized as follows.

Total contaminant concentration in < 2 mm soil (Table F-2) and < 250 um (Table F-3) found showed 4 soils contaminated with As, 3 soils contaminated with Cd, 3 soils contaminated with Cr, 8 soils contaminated with Pb, and 6 soils contaminated with Zn (Table F-4).   In some soils, large differences were found between contaminant concentration in < 2 mm and < 250 um fractions.  These differences underscore the importance of using contaminant concentration data from different size fractions when performing human or ecological risk assessments.  In this case, contaminant concentration data for the < 2 mm fraction was used for ecological assessment evaluated with plants and earthworms. Contaminant concentration data for the < 250 um fraction was used for human risk assessment evaluated through the soil ingestion pathway.

Higher concentrations in the < 250 um fraction is consistent with reaction between the soil surfaces and dissolved metal contaminant.  The finer < 250 um fraction has greater specific surface area (surface area/weight) than the < 2 mm fraction. The greater surface area in the < 250 um vs. < 2 mm soil will adsorb greater amounts of metal contaminant and result in higher contaminant concentration in the finer < 250 um fraction.  Soil fractions with different surface areas but similar metal contaminant concentrations suggests the metal contaminant may not have reacted with soil surfaces and occurs as discrete soil mineral precipitates or as chemically unweathered contaminant.

**Table F-1.** Site names, location and contaminant sources.

| Site Name | Site Location | Suspected Contaminant(s) | Suspected contaminant source(s) | Soil Type |
|---|---|---|---|---|
| MCAS Cherry Point | Cherry Point, NC | Cr, Pb | incinerator | Entisol |
| Concord Naval Weapons | Concord, CA | As | pesticide use | Vertisol |
| Deseret Chemical Depot | Tooele, UT | As | alluvial mine tailings | Aridisol |
| Hill AFB | Ogden, UT | As, Cd, Cr, Pb | Water treatment sludge drying bed | Entisol |
| Sugar Cane Farm | Hilo, HI | As | pesticide use | Andisol |
| McClellan AFB | Sacramento, CA | Cd, Cr, Pb | Wastewater treatment lagoon | Alfisol |
| NSA | Mechanicsburg, PA | Pb | lead ingot storage area | Ultisol |
| ORNL Firing Range | Oak Ridge, TN | Pb | small arms fire | Ultisol |
| Fuel Depot | Pearl City, HI | As, Pb | pesticides | Mollisol |
| Portsmouth Naval Shipyard | Kittery, ME | Pb | Lead battery cells | Inceptisol |
| Travis AFB | Fairfield, CA | Pb | small arms fire | Alfisol |
| Naval Base Point Loma | San Diego, CA | As, Pb | **??** | **??** |

**Table F-2.** Total Elemental Content† of contaminated soil (C) and reference (i.e. uncontaminated) soil (R) for the study sites. All soils are < 2 mm fraction.

| | | Study Site | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cherry Pt | | Concord | | Deseret | | Hill‡ | Hilo | | McCllelan | |
| | units | C | R | C | R | C | R | C | C | R | C | R |
| Al | g/kg | 14.7 | 9.20 | 39.0 | 37.8 | 16.8 | 25.3 | 21.7 | 25.6 | 16.7 | 40.1 | 6.66 |
| As | mg/kg | 6.89 | 1.73 | 220 | 7.84 | 438 | 11.2 | 19.5 | 660 | 21.7 | 9.88 | 6.08 |
| Ba | mg/kg | 200 | 38.6 | 250 | 241 | 529 | 191 | 164 | 292 | 38.4 | 333 | 0.119 |
| Cd | mg/kg | 18.9 | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 | 43.8 | 5.90 | 1.29 | 21.9 | 0.652 |
| Co | mg/kg | <1.0 | <1.0 | 16.5 | 17.0 | 5.19 | 6.51 | 9.12 | < 0.2 | 7.77 | 7.55 | 1.44 |
| Cr | mg/kg | 876 | 13.3 | 76.6 | 79.0 | 23.6 | 26.7 | 239 | 140 | 120.2 | 699 | 126 |
| Cu | mg/kg | 167 | <1.0 | 54.3 | 50.1 | 12.6 | 15.2 | 81.8 | 224 | 69.2 | 241 | 0.360 |
| Fe | g/kg | 10.4 | 4.11 | 39.8 | 38.2 | 17.5 | 21.2 | 28.9 | 74.3 | 45.6 | 28.2 | 0.42 |
| Mg | g/kg | 633 | 823 | 10.4 | 10.4 | 8.56 | 10.4 | 12.9 | 22.1 | 26.3 | 3.66 | <0.1 |
| Mn | mg/kg | 48.4 | 27.0 | 843 | 835 | 538 | 515 | 553 | 914 | 503 | 128 | 14.7 |
| Mo | mg/kg | 4.83 | <1.0 | <1.0 | <1.0 | 1.13 | <1.0 | 7.69 | < 2 | <2 | 2.26 | 12.0 |
| Ni | mg/kg | 77.6 | 3.49 | 91.5 | 97.9 | 16.3 | 16.9 | 40.0 | 417 | 561 | 87.0 | 59.9 |
| P | mg/kg | 6,482 | 197 | 606 | 390 | 740 | 550 | 784 | 4318 | 796 | 1068 | 25.2 |
| Pb | mg/kg | 114 | 16.7 | 22.4 | 15.8 | 18.6 | 19.5 | 51.1 | 2134 | 153 | 193 | 14.9 |
| Se | mg/kg | 2.59 | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 | <2 | <2 | <1.0 | 1.71 |
| Tl | mg/kg | <1.0 | <1.0 | <1.0 | <1.0 | 15.7 | <1.0 | <1.0 | <2 | <2 | <1.0 | 93.1 |
| V | mg/kg | 69.6 | 14.8 | 117 | 113 | 33.6 | 39.8 | 48.2 | 70.2 | 45.7 | 109 | 558 |
| Zn | mg/kg | 486 | 31.7 | 112 | 101 | 85.2 | 83.2 | 203 | 1889 | 282 | 448 | 32.0 |

† Acid Digestion (USEPA 3051a) followed by analysis using high resolution ICP OES.

‡ Hill AFB did not have a reference soil

**Table F-2 (continued).** Total Elemental Content† of contaminated soil (C) and reference (i.e. uncontaminated) soil (R) of the study sites. All soils are < 2 mm fraction.

| | Units | Mechanicsburg | | ORNL | | Pearl City | | Portsmouth | | Travis | | Point Loma§ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | R | C | R | C | R | C | R | C | R | C |
| Al | g/kg | 43.8 | 53.8 | 12.6 | 49.5 | 44.6 | 60.4 | 11.1 | 10.6 | 26.0 | 30.0 | 15.0 |
| As | mg/kg | 14.6 | 16.8 | 5.01 | 13.9 | 619 | 4.08 | 11.3 | 10.0 | 10.9 | 8.11 | 3.67 |
| Ba | mg/kg | 127 | 149 | 309 | 66.4 | 221 | 103 | 133 | 74.4 | 300 | 211 | 18.9 |
| Cd | mg/kg | <1.0 | <1.0 | <1.0 | <1.0 | 3.63 | 1.41 | 1.14 | <1.0 | <1.0 | <1.0 | <1.0 |
| Co | mg/kg | 11.1 | 15.7 | <1.0 | <1.0 | <0.2 | <0.2 | 6.30 | 37.9 | 9.13 | 9.14 | <1.0 |
| Cr | mg/kg | 39.3 | 55.8 | 16.0 | 48.0 | 185 | 233 | 10.9 | 13.7 | 42.4 | 42.8 | 22.8 |
| Cu | mg/kg | 25.4 | 18.7 | 65.0 | 13.9 | 423 | 110 | 185 | 12.3 | 1477 | 18.9 | 10.6 |
| Fe | g/kg | 29.5 | 36.5 | 11.9 | 28.1 | 118 | 92.9 | 19.8 | 10.7 | 27.1 | 24.0 | 20.8 |
| Mg | g/kg | 13.0 | 6.32 | 711 | 3.45 | 7.21 | 7.78 | 1.90 | 1.48 | 33.3 | 3.22 | 4.26 |
| Mn | mg/kg | 651 | 1126 | 88.3 | 60.8 | 1384 | 701 | 231 | 163 | 513 | 534 | 244 |
| Mo | mg/kg | 1.63 | <1.0 | <1.0 | <1.0 | 18.6 | <2 | 2.85 | <1.0 | <1.0 | <1.0 | <1.0 |
| Ni | mg/kg | 28.8 | 35.5 | 4.22 | 14.7 | 196 | 182 | 61.5 | 8.42 | 28.5 | 22.9 | 6.83 |
| P | mg/kg | 362 | 443 | 75.1 | 94.6 | 1121 | 1001 | 436 | 392 | 289 | 207 | 233 |
| Pb | mg/kg | 120 | 32.9 | 966 | 12.2 | 1466 | 13.1 | 3069 | 47.9 | 2034 | 16.9 | 8.65 |
| Se | mg/kg | <1.0 | <1.0 | <1.0 | <1.0 | <2.0 | <2.0 | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 |
| Tl | mg/kg | <1.0 | <1.0 | <1.0 | <1.0 | <2.0 | <2.0 | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 |
| V | mg/kg | 58.2 | 76.7 | 28.6 | 75.7 | 197 | 205 | 37.0 | 16.3 | 69.3 | 73.8 | 58.0 |
| Zn | mg/kg | 97.8 | 96.8 | 30.1 | 85.2 | 1804 | 133 | 500 | 59.5 | 225 | 69.9 | 60.8 |

§ Point Loma soil was not contaminated.

**Table F-3.** Total Elemental Content† of contaminated soil of the study sites. All soils are < 250 um fraction.

|    |       | Study Site | | | | | |
|----|-------|-----------|---------|---------|------|------|----------|
|    |       | Cherry Pt | Concord | Deseret | Hill | Hilo | McCllelan |
| Al | g/kg  | 23.3  | 38.8 | 14.1 | 28.6 | 32.4  | 39.9 |
| As | mg/kg | 11.4  | 222  | 521  | 21.8 | 904   | 10.4 |
| Ba | mg/kg | 308   | 254  | 602  | 214  | 468   | 285  |
| Cd | mg/kg | 82.3  | <1   | <1   | 66.0 | 7.06  | 22.1 |
| Co | mg/kg | <1    | <1   | 5.23 | 10.7 | <0.2  | 7.75 |
| Cr | mg/kg | 1456  | 85.2 | 21.1 | 369  | 155   | 593  |
| Cu | mg/kg | 276   | 62.6 | 12.6 | 114  | 339   | 232  |
| Fe | g/kg  | 17.3  | 39.9 | 17.3 | 30.9 | 70.0  | 28.9 |
| Mg | g/kg  | 1.01  | 10.4 | 7.97 | 13.6 | 21.3  | 3.64 |
| Mn | mg/kg | 80.9  | 848  | 511  | 725  | 1025  | 126  |
| Mo | mg/kg | 8.16  | <2   | <2   | 11.9 | <2    | <2   |
| Ni | mg/kg | 125   | 93.1 | 16.3 | 51.2 | 300   | 76.1 |
| P  | mg/kg | 10823 | 622  | 693  | 863  | 5528  | 856  |
| Pb | mg/kg | 189   | 23.9 | 18.7 | 77.7 | 3182  | 164  |
| Se | mg/kg | 4.10  | <2   | <2   | 2    | <2    | <2   |
| Tl | mg/kg | <2    | <2   | 18.1 | <2   | <2    | <2   |
| V  | mg/kg | 112   | 114  | 30.6 | 59.0 | 91.3  | 112  |
| Zn | mg/kg | 778   | 107  | 85.1 | 278  | 2525  | 445  |

† Acid Digestion (USEPA 3051a) followed by analysis using ICP OES.

**Table F-3 (continued).**  Total Elemental Content† of contaminated soil of the study sites.  All soils are < 250 um fraction.

|    |       | Mechanicsburg | ORNL | Pearl City | Portsmouth | Travis |
|----|-------|---------------|------|------------|------------|--------|
| Al | g/kg  | 41.8 | 14.0 | 59.8 | 14.6 | 32.4 |
| As | mg/kg | 13.0 | 4.69 | 464 | 16.6 | 11.8 |
| Ba | mg/kg | 133 | 395 | 240 | 132 | 358 |
| Cd | mg/kg | <1 | <1 | 3.46 | 1.43 | <1 |
| Co | mg/kg | 9.46 | <1 | <0.2 | 8.26 | 9.47 |
| Cr | mg/kg | 35.8 | 16.5 | 215 | 55.2 | 48.3 |
| Cu | mg/kg | 26.6 | 79.0 | 399 | 256 | 250 |
| Fe | g/kg  | 25.1 | 11.0 | 118 | 23.5 | 28.7 |
| Mg | g/kg  | 12.1 | 0.77 | 6.06 | 2.43 | 3.61 |
| Mn | mg/kg | 447 | 66.2 | 1466 | 336 | 537 |
| Mo | mg/kg | <2 | <2 | 5.94 | 4.45 | <2 |
| Ni | mg/kg | 27.6 | 5.00 | 240 | 116 | 29.9 |
| P  | mg/kg | 334 | 75.3 | 1252 | 649 | 320 |
| Pb | mg/kg | 223 | 1127 | 1616 | 4113 | 2416 |
| Se | mg/kg | <2 | <2 | <2 | <2 | <2 |
| Tl | mg/kg | <2 | 5.34 | <2 | <2 | <2 |
| V  | mg/kg | 52.4 | 25.0 | 213 | 58.5 | 77.6 |
| Zn | mg/kg | 98.5 | 34.0 | 1559 | 757 | 117 |

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**Table F-4.** Summary of metal(loid) contaminated study sites. Contaminant concentrations are in mg/kg in < 2 mm soil from study sites

| | | Ch Pt | Con-cord | Dese rt | Hill | Hilo | McCle | Mecha nic | ORNL | Pearl City | Port | Travis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| As | < 2 mm | | 220 | 438 | | 660 | | | | 619 | | |
| | <250 um | | 222 | 521 | | 904 | | | | 464 | | |
| Cd | < 2 mm | 18.9 | | | 43.8 | | 21.9 | | | | | |
| | <250 um | 82.3 | | | 66.0 | | 22.1 | | | | | |
| Cr | < 2 mm | 876 | | | 239 | | 699 | | | | | |
| | <250 um | 1456 | | | 369 | | 593 | | | | | |
| Pb | < 2 mm | 114 | | | | 2134 | 193 | 120 | 966 | 1466 | 3069 | 2034 |
| | <250 um | 189 | | | | 3182 | 164 | 223 | 1127 | 1616 | 4113 | 2416 |
| Zn | < 2 mm | 486 | | | | 1889 | 448 | | | 1804 | 500 | 225 |
| | <250 um | 778 | | | | 2525 | 445 | | | 1559 | 757 | 117 |

Select soil properties, known to affect soil contaminant bioavailability and plant growth, were determined for both soil size fractions. Soil properties < 2 mm soil (Table F-5) and < 250 um (Table F-6) are summarized as follows. There was a wide range in properties for contaminated soils (Table F-7). The best attempt to obtain control soils with properties similar to contaminated soils was made. However, the source of the contamination did alter key soil properties (i.e., soil pH, reactive oxide content, carbon content) for some of the contaminated soils. In these cases, differences in key soil properties may alter contaminant bioavailability and affect plant dry matter production. The low soil pH (<5.0) of the ORNL and the McClellan soils will likely results in aluminum phytotoxicity and prevent plant bioassays from being conducted on these soils.

There was a wide range in properties of the < 250 um soil fraction used to assess human risk from soil ingestion (Table F-8). Summary statistics of properties of < 250 um soil are listed in Table F-9.

**Table F-5.** Select Soil Properties of contaminated soil (C) and reference (i.e. uncontaminated) soil (R). All soils are < 2 mm fraction.

| | units | Cherry Pt C | Cherry Pt R | Concord C | Concord R | Deseret C | Deseret R | Hill C | Hilo C | Hilo R | McCllelan C | McCllelan R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Soil pH, water | | 5.50 | 7.43 | 6.67 | 6.34 | 9.28 | 7.84 | 7.22 | 5.88 | 4.71 | 4.31 | 6.66 |
| Soil pH, $CaCl_2$ | | 5.01 | 6.96 | 6.15 | 5.89 | 7.49 | 6.91 | 7.08 | 5.74 | 4.73 | 4.32 | 6.08 |
| EC | dS/m | 0.892 | 0.353 | 0.111 | 0.189 | 0.544 | 0.480 | 0.989 | 0.820 | 1.53 | 0.276 | 0.119 |
| Alox | mg/kg | 6061 | 909 | 1522 | 1672 | 786 | 1207 | 1175 | 21344 | 5917 | 2175 | 487 |
| Feox | mg/kg | 7506 | 797 | 3664 | 4519 | 863 | 681 | 956 | 25678 | 7535 | 4805 | 804 |
| Mnox | mg/kg | 32.2 | <25 | 641 | 659 | 313 | 381 | 333 | 484 | 85.7 | <25 | 125 |
| Org C | % | 3.71 | 0.758 | 3.13 | 2.17 | 0.645 | 0.792 | 1.50 | 7.77 | 5.69 | 4.36 | 0.360 |
| Total C | % | 4.54 | 1.94 | 3.04 | 2.13 | 2.32 | 1.52 | 2.66 | 8.44 | 5.50 | 4.66 | 0.42 |
| CEC | $cmol_c$/kg | 9.14 | 3.94 | 27.9 | 27.7 | 8.37 | 13.4 | 11.0 | 17.1 | 10.1 | 13.4 | 12.0 |
| Sand | % | 79.7 | 80.0 | 18.4 | 19.9 | 36.6 | 27.5 | 52.3 | 61.1 | 72.3 | 25.7 | 59.9 |
| Silt | % | 13.5 | 12.2 | 40.9 | 44.3 | 54.7 | 53.2 | 31.3 | 25.3 | 17.8 | 50.2 | 25.2 |
| Clay | % | 6.8 | 7.8 | 40.7 | 35.8 | 8.7 | 19.3 | 16.4 | 7.8 | 2.6 | 24.1 | 14.9 |

Soil pH (water): pH measured in 1:1 soil:deionized water suspension
Soil pH ($CaCl_2$): pH measured in 1:2 soil: 0.01 M $CaCl_2$ suspension
EC: electrical conductivity measured in 1:1 soil:deionized water suspension
Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction

**Table F-5 (continued).**

| | | Mechanicsburg | | ORNL | | Pearl City | | Portsmouth | | Travis | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | R | C | R | C | R | C | R | C | **R** |
| Soil pH, water | | 8.04 | 7.46 | 4.1 | 3.81 | 7.34 | 7.65 | 6.2 | 6.2 | 7.04 | 6.02 |
| Soil pH, CaCl$_2$ | | 7.04 | 7.12 | 3.53 | 3.14 | 7.28 | 7.47 | 6.04 | 5.72 | 6.46 | 5.63 |
| EC | dS/m | 0.209 | 0.291 | 0.184 | 0.152 | 0.995 | 0.929 | 0.089 | 0.183 | 0.247 | 0.261 |
| Alox | mg/kg | 1615 | 2050 | 388 | 851 | 3502 | 2046 | 3764 | 4149 | 799 | 885 |
| Feox | mg/kg | 1407 | 2492 | 507 | 798 | 44900 | 1977 | 5758 | 2682 | 3088 | 4569 |
| Mnox | mg/kg | 290 | 944 | 27.4 | <25 | 1014 | 492 | 124 | 70.1 | 405 | 547 |
| Org C | % | 0.640 | 1.22 | 0.326 | 0.222 | 2.34 | 0.29 | 1.64 | 1.44 | 1.09 | 1.32 |
| Total C | % | 4.49 | 1.43 | 0.38 | 0.17 | 3.33 | 2.01 | 2.57 | 1.72 | 1.22 | 1.39 |
| CEC | cmol$_c$/kg | 9.74 | 9.58 | 2.79 | 7.90 | 25.9 | 39.4 | 2.73 | 2.68 | 17.3 | 10.8 |
| Sand | % | 29.9 | 9.90 | 45.7 | 9.0 | 48.7 | 54.7 | 89.0 | 86.5 | 47.6 | 29.9 |
| Silt | % | 36.6 | 50.0 | 36.5 | 33.4 | 29.2 | 26.9 | 8.5 | 9.6 | 26.3 | 44.3 |
| Clay | % | 33.5 | 40.1 | 17.8 | 57.6 | 22.1 | 18.4 | 2.5 | 3.9 | 26.1 | 25.8 |

Soil pH (water): pH measured in 1:1 soil:deionized water suspension
Soil pH (CaCl$_2$): pH measured in 1:2 soil: 0.01 M CaCl$_2$ suspension
EC: electrical conductivity measured in 1:1 soil:deionized water suspension
Alox, Feox, Mnox:  reactive oxide fraction measured using acid ammonium oxalate extraction

**Table F-6. Select Properties of ESTCP Contaminated soils (C) and Reference (uncontaminated) soils (R). All soils are < 250 μm fraction.**

|  | units | Cherry Pt | | Concord | | Deseret | | Hill | Hilo | | McCllelan | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  | C | R | C | R | C | R | C | C | R | C | R |
| **Alox** | mg/kg | 10897 | 988 | 1746 | 1765 | 747 | 1251 | 1548 | 28692 | none | 3415 | 650 |
| **Feox** | mg/kg | 13216 | 821 | 4207 | 4752 | 1037 | 763 | 1358 | 30671 | none | 6248 | 1482 |
| **Mnox** | mg/kg | 54.3 | <25 | 634 | 621 | 293 | 224 | 413 | 635 | none | <25 | 125 |
| **Org C** | % | 5.94 | 0.97 | 2.59 | 1.79 | 0.48 | 0.73 | 2.02 | 9.42 | none | 4.56 | 0.52 |
| **Total C** | % | 7.71 | 1.62 | 3.18 | 2.11 | 2.00 | 1.33 | 3.31 | 10.6 | none | 4.42 | 0.548 |
| **CBD Fe** | mg/kg | 10824 | --- | 12749 | --- | 6044 | --- | 4530 | 29606 | --- | 6030 | --- |

Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction

CBD Fe: citrate-bicarbonate-dithionite extractable Fe

**Table F-6 (continued). Select Properties of ESTCP Contaminated soils (C) and Reference (uncontaminated) soils (R). All soils are < 250 μm fraction.**

|         |        | Mechanicsburg | | ORNL | | Pearl City | | Portsmouth | | Travis | |
|---------|--------|------|------|------|-------|-------|------|-------|------|-------|------|
|         |        | C    | R    | C    | R     | C     | R    | C     | R    | C     | R    |
| **Alox**    | **mg/kg** | 2182 | 2160 | 473  | 828   | 4155  | none | 5739  | 6481 | 1016  | 902  |
| **Feox**    | **mg/kg** | 1993 | 2692 | 556  | 786   | 52796 | none | 9177  | 4291 | 3630  | 4697 |
| **Mnox**    | **mg/kg** | 257  | 914  | 32.7 | <25   | 1192  | none | 178   | 104  | 402   | 424  |
| **Org C**   | **%**     | 0.83 | 1.37 | 0.33 | 0.22  | 3.22  | none | 2.78  | 2.41 | 1.12  | 1.16 |
| **Total C** | **%**     | 4.41 | 1.69 | 0.365| 0.205 | 3.21  | none | 3.07  | 2.78 | 1.28  | 1.36 |
| **CBD Fe**  | **mg/kg** | 16348| ---  | 8715 | ---   | 31795 | ---  | 11992 | ---  | 11247 | ---  |

Alox, Feox, Mnox:  reactive oxide fraction measured using acid ammonium oxalate extraction

CBD Fe:  citrate-bicarbonate-dithionite extractable Fe

**Table F-7.** Summary Statistics of Soil Properties of contaminated soils. All soils are < 2 mm fraction.

| Property | Units | Min | Max | Mean | Median | 90th percentile |
|---|---|---|---|---|---|---|
| Soil pH, water | | 4.10 | 9.28 | 6.51 | 6.67 | 8.04 |
| Soil pH, CaCl$_2$ | | 3.53 | 7.49 | 6.10 | 6.15 | 7.28 |
| EC | dS/m | 0.089 | 0.995 | 0.487 | 0.276 | 0.989 |
| Alox | mg/kg | 388 | 21344 | 3921 | 1615 | 6061 |
| Feox | mg/kg | 507 | 44900 | 9012 | 3664 | 25678 |
| Mnox | mg/kg | 27.4 | 1014 | 366 | 323 | 678 |
| Org C | % | 0.326 | 7.77 | 2.47 | 1.64 | 4.36 |
| Total C | % | 0.38 | 8.44 | 3.42 | 3.04 | 4.66 |
| CEC | cmol$_c$/kg | 2.73 | 27.9 | 13.2 | 11.0 | 25.9 |
| Sand | % | 18.4 | 89.0 | 48.6 | 47.6 | 79.7 |
| Silt | % | 8.5 | 54.7 | 32.1 | 31.3 | 50.2 |
| Clay | % | 2.5 | 40.7 | 18.8 | 17.8 | 33.5 |

**Table F-8.** Select Soil Properties of contaminated soils.  All soils are < 250 μm fraction

|         | units | Ch Pt | Concord | Deseret | Hill | Hilo  | McCllelan |
|---------|-------|-------|---------|---------|------|-------|-----------|
| Alox    | mg/kg | 10897 | 1746    | 747     | 1548 | 28692 | 3415      |
| Feox    | mg/kg | 13216 | 4207    | 1037    | 1358 | 30671 | 6248      |
| Mnox    | mg/kg | 54.3  | 634     | 293     | 413  | 635   | <25       |
| Org C   | %     | 5.94  | 2.59    | 0.48    | 2.02 | 9.42  | 4.56      |
| Total C | %     | 7.71  | 3.18    | 2.00    | 3.31 | 10.6  | 4.42      |
| CBD Fe  | mg/kg | 10824 | 12749   | 6044    | 4530 | 29606 | 6030      |

Alox, Feox, Mnox:  reactive oxide fraction measured using acid ammonium oxalate extraction
CBD Fe:  citrate-bicarbonate-dithionite extractable Fe.

**Table F-8 (continued).**  Select Soil Properties of contaminated soils.  All soils are < 250 μm fraction

|         |       | Mechanicsburg | ORNL  | Pearl City | Portsmouth | Travis |
|---------|-------|---------------|-------|------------|------------|--------|
| Alox    | mg/kg | 2182          | 473   | 4155       | 5739       | 1016   |
| Feox    | mg/kg | 1993          | 556   | 52796      | 9177       | 3630   |
| Mnox    | mg/kg | 257           | 32.7  | 1192       | 178        | 402    |
| Org C   | %     | 0.83          | 0.33  | 3.22       | 2.78       | 1.12   |
| Total C | %     | 4.41          | 0.365 | 3.21       | 3.07       | 1.28   |
| CBD Fe  | mg/kg | 16348         | 8715  | 31795      | 11992      | 11247  |

Alox, Feox, Mnox:  reactive oxide fraction measured using acid ammonium oxalate extraction
CBD Fe:  citrate-bicarbonate-dithionite extractable Fe.

**Table F-9.** Summary Statistics of Soil Properties of contaminated soils.  All soils are < 250 μm fraction

|         |       | Minimum | Maximum | Mean  | Median | 90th percentile |
|---------|-------|---------|---------|-------|--------|-----------------|
| Alox    | mg/kg | 473     | 28692   | 5510  | 2182   | 10897           |
| Feox    | mg/kg | 556     | 52796   | 11354 | 4207   | 30671           |
| Mnox    | mg/kg | 32.7    | 1192    | 409   | 347    | 691             |
| Org C   | %     | 0.33    | 9.42    | 3.03  | 2.59   | 5.94            |
| Total C | %     | 0.365   | 10.6    | 3.95  | 3.21   | 7.71            |
| CBD Fe  | mg/kg | 4530    | 31795   | 13625 | 11247  | 29606           |